Pharmaceutica Analytica Acta

Wang et al., Pharm Anal Acta 2015, 6:3
http://dx.doi.org/10.4172/2153-2435.1000345

Research Article Open Access

Simultaneous Quantitative Determination of Nine Bufadienolides in Traditional Chinese Medicinal Toad Skin from Different Regions of China by High-Performance Liquid Chromatography – Photodiode Array Detection

Si-Wang Wang*, Lin-Rui Duan, Wei Cao, Yan-Hua Xie, Jia-Ni Yuan, Hua Li and Xiao-Kai Zhang

Department of Natural Medicine, School of Pharmacy, Fourth Military Medical University, 169 West Changle Road, Xi'an 710032, Shaanxi, China

Abstract

Toad skin is a traditional Chinese medicine for the treatment of various tumors. The major active components in toad skin are bufadienolides. In this paper, a simple, accurate and reliable method for the simultaneous separation and determination of nine active components (gamabufotalin, arenobufagin, telocinobufagin, desacetylcinobufotalin, bufotalin, cinobufotalin, bufalin, cinobufagin and resibufogeni) in toad skin was developed using High-Performance Liquid Chromatography (HPLC) coupled with Photodiode Array Detection (PDA) detection. The chromatographic separation was performed on a SinoChrom ODS-BP C_{18} column with gradient elution using acetonitrile and 0.1% acetic acid-0.5% potassium dihydrogen phosphate aqueous solution at a flow rate of 0.8 mL min^{-1}. All compounds showed good linearity in a wide concentration range with the values of r^2 higher than 0.9994, and their limits of detection were at the range of 0.06-0.10 μg mL^{-1}. The separation and identification of the nine bufadienolides in this paper may provide important experimental data for further research and application of toad skin.

Keywords: HPLC-PDA; Bufadienolides; Toad skin; Traditional Chinese medicine; Quantitative determination

Introduction

Toad skin is the dry epidermis of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider which is separated from internal organs, muscles and bones. The earliest record of toad skin appeared in Tang Dynasty [1]. Thousands of years ago, ancient Chinese had found the way to extract secretions from toad skin; these secretions were denominated *Venenum Bufonis*, and were widely used in China as an anodyne, cardiotonic, antimicrobial, local anesthetic, and antineoplastic agent [2].

In recent years, more and more researchers have focused on the potential antitumor activity of toad skin [3-6]. Bufadienolides, such as bufalin, cinobufagin, resibufogenin and telocinobufagin are the major active compounds derived from the toad skin [7,8]. Numerous *in vitro* and *in vivo* data have suggested that these compounds exhibit a significant antitumor activity, including the inhibition of cell proliferation, induction of cell differentiation, induction of apoptosis, disruption of the cell cycle, inhibition of cancer angiogenesis, reversal of multi-drug resistance, and regulation of the immune response [9]. Cinobufagin has been approved by the Chinese State Food and Drug Administration (SFDA) (ISO9002) and been widely used to treat patients with various cancers at oncology clinics in China [10]. A total of 39 bufadienolides have been reported [11] and 11 have been isolated, namely, resibufogenin, cinobufagin, bufalin, telocinobufagin, bufotalin, desacetylcinobufotalin, hellebrigenin, arenobufagi, gamabufotalin, 11β-hydroxylresibufogenin and cinobufotalin [12].

Currently, only a few analytical methods have been reported to determine the active components in toad skin and these reports are simply confined to 3 bufadienolides at most [13,14]. Up to now, no studies on quantitative determination of multiple active components of toad skin in a single running have been reported. Although extensive research has been conducted on the active components of *Venenum Bufonis* [15-17], great differences in chemical constituents have been observed between *Venenum Bufonis* and toad skin. Compared with those in *Venenum Bufonis*, more types and less quantity of bufadienolides have been reported in toad skin [18]. At the same time, the variation of environments and climates of different regions and the diverse ways of collection, processing and storage of the toad skin may result in different bufadienolides quantities separated from the toad skin from different regions. Therefore, it is meaningful to investigate the active components in toad skin from different regions.

To establish a standard and quality control for bufadienolides quantities separated from the toad skin from different regions, we collected and compared 10 samples of toad skin from different regions of China and an HPLC-UV method was developed for the simultaneous quantitative determination of 9 active components contained in toad skin (gamabufotalin, arenobufagin, telocinobufagin, desacetylcinobufotalin, bufotalin, cinobufotalin, bufalin, cinobufagin and resibufogenin). Our findings showed that this method is simple, accurate, reliable and particularly suitable for the routine analysis of toad skin and its quality control.

***Corresponding author:** Si-wang Wang , Department of Natural Medicine, School of Pharmacy, Fourth Military Medical University, 169 West Changle Road, Xi'an 710032, Shaanxi, China, Tel: +86 29 8322 4790; fax: +86 29 8477 4748; E-mail: siwangwangyys@163.com

Received December 29, 2014; **Accepted** March 03, 2015; **Published** March 10, 2015

Citation: Wang SW, Duan LR, Cao W, Xie YH, Yuan JN, et al. (2015) Simultaneous Quantitative Determination of Nine Bufadienolides in Traditional Chinese Medicinal Toad Skin from Different Regions of China by High-Performance Liquid Chromatography–Photodiode Array Detection. Pharm Anal Acta 6: 345. doi:10.4172/2153-2435.1000345

Experimental

Chemicals and reagents

Standards of gamabufotalin, arenobufagin, telocinobufagin, desacetylcinobufotalin, bufotalin, cinobufotalin, bufalin, cinobufagin and resibufogenin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). The structures of the nine bufadienolides are presented in figure 1.

Acetonitrile (MeCN) (HPLC-grade) and methanol (MeOH) (HPLC-grade) were purchased from Honeywell (Muskegon, MI, USA). Acetic acid (HOAc) (HPLC-grade) was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). The ultrapure water was prepared from Millipore water purification system (Milford, MA, USA) and filtered with a 0.22 μm membrane. Phosphoric acid (H_3PO_4) and potassium dihydrogen phosphate (KH_2PO_4) used in the mobile phase were of AR-grade.

Ten samples of toad skin were purchased from the herbal market or local pharmacy in the following different regions in China: Jinan, Shandong Province (1 batch, NO. S-1); Anguo, Hebei Province (2 batches, NO. S-2, S-3); Nanyang, Henan Province (2 batches, NO. S-4 S-5); Bozhou, Anhui Province (2 batches, NO. S-6 S-7) and Xian, Shaanxi Province (3 batches, NO. S-8 S-9 S-10).

Preparation of standard solutions

A standard stock solution containing the 9 components (gamabufotalin 200 μg ml^{-1}, arenobufagin 100 μg ml^{-1}, telocinobufagin 150 μg ml^{-1}, desacetylcinobufotalin 150 μg ml^{-1}, bufotalin 240 μg ml^{-1}, cinobufotalin 180 μg ml^{-1}, bufalin 300 μg ml^{-1}, cinobufagin 400 μg ml^{-1} and resibufogenin 450 μg ml^{-1}) was prepared in chromatographic pure methanol and stored away from light at 4°C. Working standard solutions containing the 9 compounds were prepared by appropriate dilution of the stock solution.

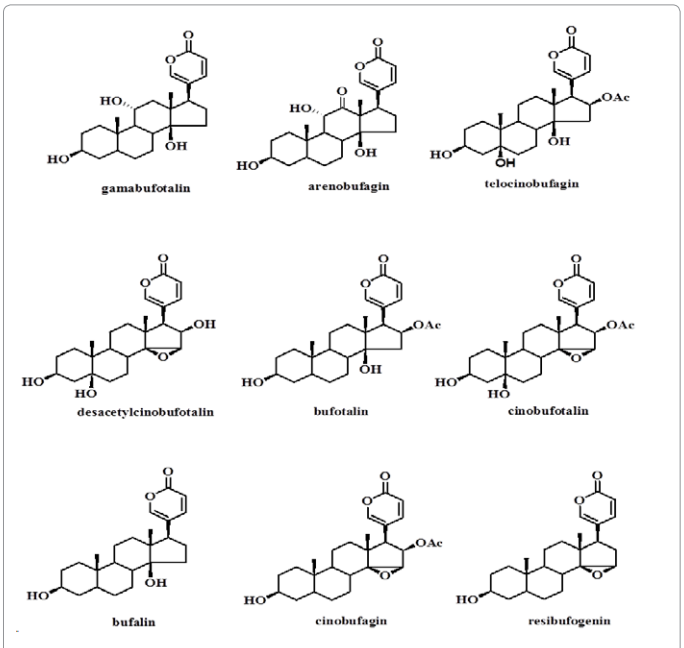

Figure 1: Chemical structures of 9 bufadienolides contained in toad skin.

Preparation of sample solutions

1 g of each of the 10 powdered samples of toad skin was extracted with methanol (20 mL HPLC-grade) by recirculate for 1 h at 70°C, and then methanol was added to make up for a loss due to volatilization. The resulting extract was centrifuged for 15 min at 12,000×g and was filtered with a 0.45 μm microporous membrane prior to analysis. Aliquot (20 μL) of sample solution was injected into the HPLC system for analysis.

Apparatus and chromatographic conditions

The analyses were performed using a Shimadzu Prominence LC-20A HPLC system (Shimadzu, Kyoto, Japan) equipped with a LC-20AD quaternary pump, a SPD-M20A PDA detector, a SIL-20AC HT auto sampler, a CTO-20A thermostat compartment and a LC-solution software. The samples were separated on a SinoChrom ODS-BP C_{18} column (4.6 mm×250 mm 5 μm Yilite, Dalian, China) and a C_{18} guard column was used before the analytical column. The mobile phase consisted of MeCN and 0.1% HOAc-0.5% KH_2PO_4 aqueous solution (adjusted to pH 2.4 with H_3PO_4). A multistep gradient program was used as follows: 8% MeCN (0 min), 30% MeCN (20 min), 40% MeCN (45 min), 50% MeCN (70 min), 40% MeCN (75 min) and 8% MeCN (80 min). The flowrate was 0.8 mL min^{-1}, column temperature was maintained at 30°C and detection wavelength was recorded at 296 nm.

Result and Discussion

Optimization of HPLC conditions

In order to achieve the best separation, various factors were examined, including chromatographic column, mobile phase, elution mode, and detection wavelength as well as flow rate and column temperature. Ultimate XB C_{18} column, Phenomenex Luna 5u C_{18} column, Yilite Hypersil BDS C_{18} column and Yilite SinoChrom ODS-BP C_{18} column were employed. Compared with methanol, acetonitrile combined with phosphoric acid and potassium dihydrogen phosphate buffer solution remarkably improved the separation of the major constituents in toad skin. In addition, the addition of acetic acid had substantial effects on the selectivity and efficiency of some compounds. Under different gradient elution modes, various kinds of pH, flow rate and column temperature were also compared. The detection wavelength was selected according to the characteristic UV profile, gamabufotalin, arenobufagin, telocinobufagin, desacetylcinobufotalin, bufotalin, cinobufotalin, bufalin, cinobufagin and resibufogenin at 300.1, 299.0, 296.6, 295.4, 295.4, 301.3, 295.4, 300.1 and 296.6 nm, respectively. The ultraviolet absorption spectrums are presented in figure 2. Nine bufadienolides compounds from toad skin were detected under the current HPLC condition and were compared with the bufadienolides standards. After many tests, SinoChrom ODS-BP C_{18} column with the acetonitrile and 0.1% acetic acid-0.5% potassium dihydrogen phosphate aqueous solution (adjusted to pH 2.4 with phosphoric acid) using gradient elution was selected for the simultaneous separation and determination. The representative HPLC chromatograms of the toad skin samples and authentic standards are presented in figure 3.

Method validation

Calibration curves and the limit of detection: All calibration curves were plotted based on linear regression analysis of the integrated peak areas (y) versus concentrations (x, μg mL^{-1}) of the 9 marker constituents in the standard solution at seven different concentrations. The regression equations, correlation coefficients and linear ranges for the analysis of the 9 marker constituents are shown in table 1.

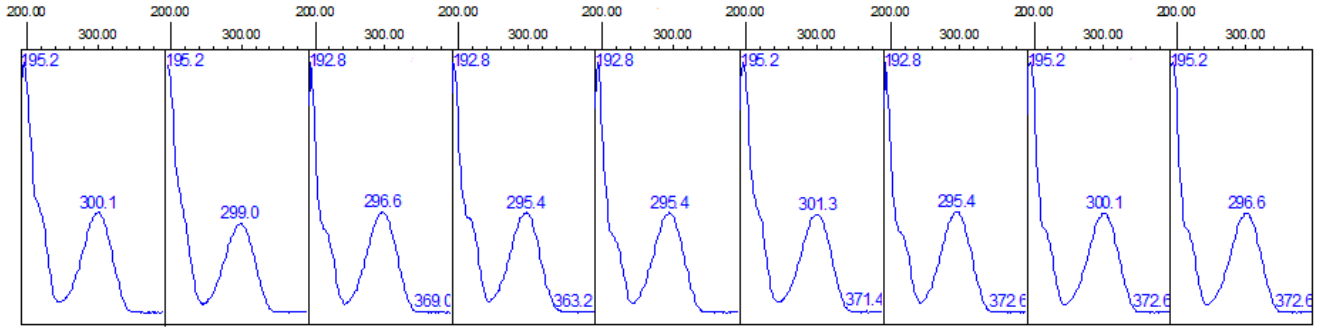

Figure 2: Ultraviolet absorption spectrums of nine bufadienolides.

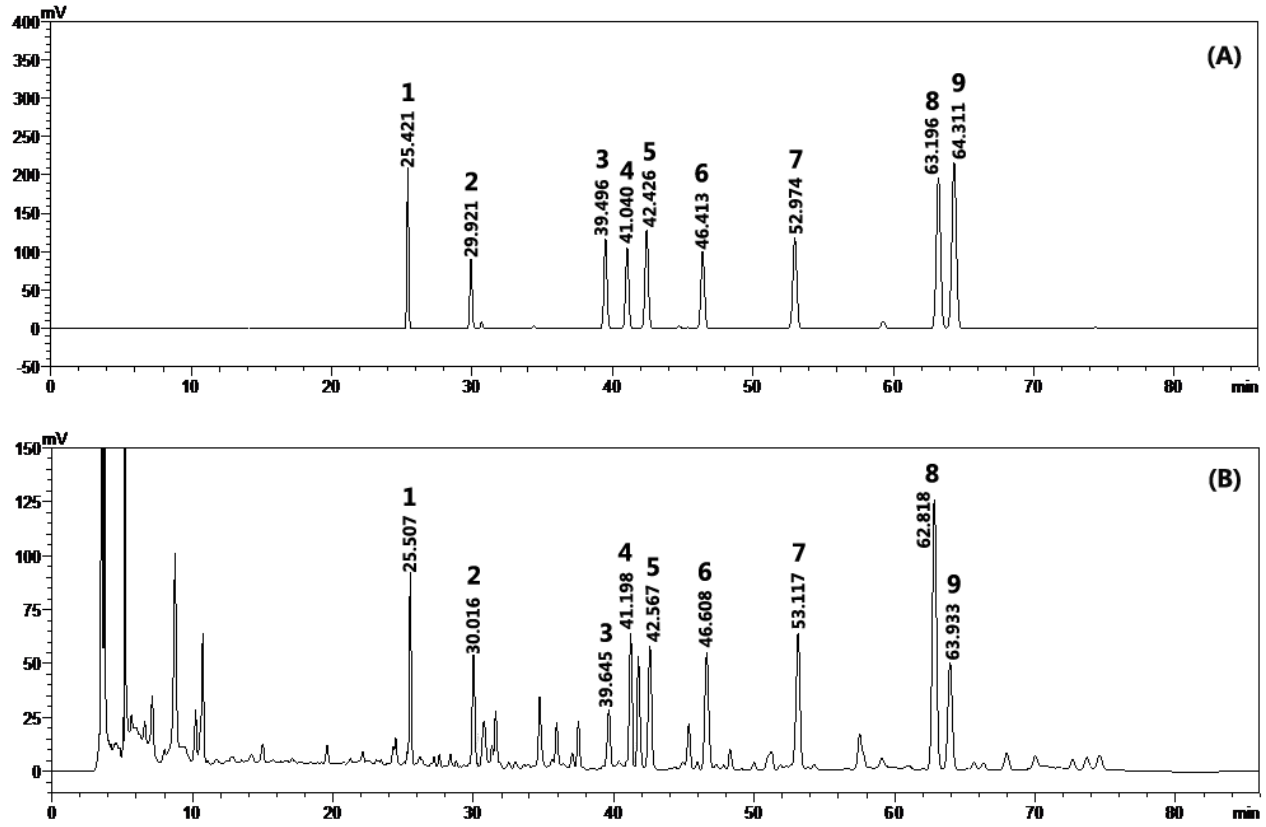

Figure 3 Typical chromatograms of standard mixture (A) and toad skin samples (B) at 296 nm.

(1) gamabufotalin; (2) arenobufagin; (3) telocinobufagin; (4) desacetylcinobufotalin; (5) bufotalin; (6) cinobufotalin; (7) bufalin; (8) cinobufagin; (9) resibufogenin.

The limit of detection value (LOD) was calculated as the amount of the injected sample which gave a signal-to-noise ratio of 3 (S/N= 3). The LOD values of the method for the 9 components are also listed (Table 1).

Precision and accuracy: The precision and the accuracy of the assay were evaluated with standard solutions at low, medium and high concentrations and measured by the Relative Standard Deviation (RSD). The intra- and inter-day precision was determined during a single day and on five consecutive days, respectively. Results are summarized (Table 2). The intra- and inter-day precision calculated as the RSD were less than 0.8%. The accuracies of nine bufadienolides were within the range of 99.67-100.69%.

Recovery: Three different concentrations (low, medium and high) of authentic standards were added into samples (NO. S-1). The resultant samples were processed and analyzed. The quantity of each analyte was subsequently obtained from the corresponding calibration curve. Recovery of all 9 tested bioactive constituents was within the range of 98.21-101.69%, with an RSD of between 0.03% and 1.23% (n=3). The corresponding values are shown in table 3.

Constituent	Regression equation[a]	Correlation coefficient (r^2)	Linearity range(μg mL^{-1})	LOD (μg mL^{-1})
gamabufotalin	y=9213.8x + 14106	0.9994	3.13-200	0.08
arenobufagin	y=10915x + 5079.1	0.9998	1.56-100	0.07
telocinobufagin	y=12147x + 12169	0.9996	2.34-150	0.06
desacetylcinobufotalin	y=11225x + 10293	0.9997	2.34-150	0.07
bufotalin	y=9079.4x + 14123	0.9996	3.75-240	0.09
cinobufotalin	y=10229x + 8819.8	0.9998	2.81-180	0.08
bufalin	y=7979.2x + 13995	0.9996	4.69-300	0.10
cinobufagin	y=10636x + 23504	0.9997	6.25-400	0.07
resibufogenin	y=10860x + 28880	0.9998	7.03-450	0.07

[a]y: peak area of components; x: concentration of components.

Table 1: Regression equation, linear range and LODs of the developed method.

Components	Nominal concentration (μg mL^{-1})	Precision					
		Intra-day (n =5)			Inter-day (n =5)		
		Mean ± SD (μg mL^{-1})	Accuracy (%)	RSD (%)	Mean ± SD (μg mL^{-1})	Accuracy (%)	RSD (%)
gamabufotalin	6.25	6.23 ± 0.03	99.67	0.47	6.24 ± 0.01	99.86	0.21
	25	25.08 ± 0.01	100.3	0.04	24.95 ± 0.09	99.78	0.37
	100	100.04 ± 0.06	100.04	0.06	100.00 ± 0.45	100	0.45
arenobufagin	3.13	3.13 ± 0.00	100.09	0.15	3.15 ± 0.03	100.69	0.8
	12.5	12.55 ± 0.02	100.42	0.16	12.50 ± 0.09	99.99	0.75
	50	50.15 ± 0.09	100.3	0.18	50.10 ± 0.33	100.2	0.67
telocinobufagin	4.69	4.68 ± 0.01	99.86	0.27	4.69 ± 0.03	99.97	0.65
	18.75	18.75 ± 0.04	100.02	0.23	18.76 ± 0.10	100.05	0.52
	75	75.13 ± 0.08	100.18	0.11	74.98 ± 0.33	99.97	0.44
desacetylcinobufotalin	4.69	4.68 ± 0.01	99.82	0.19	4.69 ± 0.04	100.11	0.77
	18.75	18.75 ± 0.06	100	0.33	18.72 ± 0.06	99.85	0.35
	75	75.05 ± 0.07	100.07	0.09	74.98 ± 0.35	99.98	0.46
bufotalin	7.5	7.49 ± 0.01	99.86	0.09	7.52 ± 0.04	100.31	0.58
	30	30.05 ± 0.04	100.16	0.14	29.99 ± 0.20	99.98	0.68
	120	120.01 ± 0.04	100.01	0.03	119.80 ± 0.55	99.84	0.46
cinobufotalin	5.63	5.61 ± 0.01	99.67	0.13	5.62 ± 0.01	99.76	0.16
	22.5	22.50 ± 0.01	100.01	0.06	22.52 ± 0.01	100.1	0.06
	90	90.05 ± 0.06	100.06	0.06	90.07 ± 0.28	100.08	0.31
bufalin	9.38	9.37 ± 0.01	99.94	0.13	9.36 ± 0.04	99.77	0.47
	37.5	37.54 ± 0.05	100.11	0.13	37.55 ± 0.08	100.12	0.2
	150	150.10 ± 0.07	100.06	0.05	150.23 ± 0.42	100.15	0.28
cinobufagin	12.5	12.49 ± 0.03	99.94	0.26	12.56 ± 0.08	100.45	0.63
	50	50.01 ± 0.04	100.02	0.08	50.14 ± 0.15	100.28	0.29
	200	200.00 ± 0.02	100	0.01	200.22 ± 0.32	100.11	0.16
resibufogenin	14.06	14.06 ± 0.04	99.97	0.3	14.08 ± 0.04	100.18	0.28
	56.25	56.24 ± 0.06	99.98	0.11	56.29 ± 0.21	100.08	0.37
	225	224.86 ± 0.08	99.94	0.04	225.23 ± 0.82	100.1	0.37

Table 2: Precision and accuracy of the developed method.

Reproducibility and stability: For reproducibility test, five different samples from a same batch of toad skin (NO. S-1) was analyzed, and the RSD values of the peak area and retention times were no more than 2.06% and 0.15%, respectively. For stability, the same sample solution was analyzed every 12 h in 3 days at the room temperature. The RSD values of the peak area and retention times were no more than 3.94% and 0.21%, respectively. The results of reproducibility and stability test showed that the method manifested good reproducibility and the solution was considered to be stable within 72 h.

Sample analysis

With the newly established method, 9 marker constituents in 10 different batches of toad skin were determined. As shown in figure 3, table 4 under the analytical conditions, the 9 marker constituents (gamabufotalin, arenobufagin, telocinobufagin, desacetylcinobufotalin, bufotalin, cinobufotalin, bufalin, cinobufagin and resibufogenin) in toad skin could be sufficiently resolved and separated. The analysis results notably indicate that the contents of the 9 bufadienolides components in toad skin from different regions of China are very

different. The main difference of the main active component content may result from the variation of environments and climates of these regions, and from the varied ways of collection, processing and storage of the toad skin.

Conclusion

In this study, 10 samples of toad skin from different regions of China were analyzed and 9 bufadienolides compounds were simultaneously identified in the methanolic extract of the different samples of toad skin. This is the first report of the simultaneous determination of 9 major active components in toad skin by high-performance liquid chromatography, coupled with photodiode array detection. The analytical method we used is sensitive, accurate and reliable, which is suitable for the routine analysis and quality control of toad skin form different regions of China.

Components	Sample contents (μg)	Add quantity (μg)	Measured contents (μg, mean ± SD)	Recovery (%)	RSD (%)
gamabufotalin	409.69	328	743.23 ± 1.00	101.69	0.13%
		410	818.71 ± 5.72	99.76	0.70%
		492	892.90 ± 8.53	98.21	0.96%
arenobufagin	144.21	116	258.94 ± 1.53	98.91	0.59%
		145	289.14 ± 3.00	99.95	1.04%
		174	317.44 ± 0.94	99.56	0.30%
telocinobufagin	69.22	56	125.43 ± 0.44	100.38	0.35%
		70	140.00 ± 0.91	101.12	0.65%
		84	152.85 ± 1.04	99.56	0.68%
desacetylcinobufotalin	410.23	328	737.35 ± 5.53	99.73	0.75%
		410	813.20 ± 3.22	98.29	0.40%
		492	901.90 ± 6.34	99.93	0.70%
bufotalin	550.17	440	987.98 ± 5.28	99.5	0.53%
		550	1100.71 ± 9.70	100.1	0.88%
		660	1213.61 ± 4.45	100.52	0.37%
cinobufotalin	128.42	102	229.02 ± 0.85	98.24	0.37%
		128	257.68 ± 0.81	100.99	0.31%
		154	279.62 ± 1.82	98.44	0.65%
bufalin	1619.28	1296	2912.44 ± 11.29	99.78	0.39%
		1620	3213.43 ± 10.98	98.4	0.34%
		1944	3552.52 ± 4.07	99.45	0.11%
cinobufagin	710.31	568	1274.41 ± 15.65	99.31	1.23%
		710	1414.55 ± 3.78	99.19	0.27%
		852	1557.59 ± 18.10	99.45	1.16%
resibufogenin	2332.81	1864	4184.88 ± 11.26	99.36	0.27%
		2330	4659.78 ± 51.33	99.87	1.10%
		2796	5115.34 ± 1.45	99.52	0.03%

Table 3: Recovery of the developed method.

References

1. Xiao PG (2002) Modern Chinese Materia Medica, vol. IV. Chemical Industry Press, Beijing.
2. National Commission of Chinese Pharmacopoeia (2010) Pharmacopoeia of the People's Republic of China, China Medical Science and Technology Press, Beijing.
3. Giri B, Debnath A, Saha A, Biswas AK, Dasgupta SC, et al. (2006) Antiproliferative, cytotoxic and apoptogenic activity of Indian toad (Bufo melanostictus, Schneider) skin extract on U937 and K562 cells. Toxicon 48:388-400.
4. Gomes A, Giri B, Kole L, Saha A, Debnath A, et al. (2007) A crystalline compound (BM-ANF1) from the Indian toad (Bufo melanostictus, Schneider) skin extract, induced antiproliferation and apoptosis in leukemic and hepatoma cell line involving cell cycle proteins. Toxicon 50:835-849.
5. Kim JS, Jeong TY, Cho CK, Lee YW, Yoo HS (2010) Antitumor Effect of Skin of Venenum Bufonis in a NCI-H460 Tumor Regression Model. Journal of Acupuncture and Meridian Studies 3: 181-187.
6. Giri B, Alam A, Mukherjee S, Bhattacharjee P, Gomes A, et al. (2011) Anticancer activity of a low immunogenic protein toxin (BMP1) from Indian toad (Bufo melanostictus, Schneider) skin extract. Toxicon 58: 85-92.
7. Resck IS, Cavalcanti BC, Moraes MO, Ferreira JRO, Rodrigues FAR et al. (2010) Cytotoxic profile of natural and some modified bufadienolides from toad Rhinella schneideri parotoid gland secretion. Toxicon 56: 339-348.
8. Li AY, Zhao L, Inagaki Y, Kokudo N, Tang W et al (2010) Cinobufacini, an aqueous extract from Bufo bufo gargarizans Cantor, induces apoptosis through a mitochondria-mediated pathway in human hepatocellular carcinoma cells. Journal of Ethnopharmacology 128: 654-661.
9. Qi FH, Inagaki Y, Kokudo N, Tamura S, Nakata M, et al. (2011) Antitumor activity of extracts and compounds from the skin of the toad Bufo bufo gargarizans Cantor. International Immunopharmacology 11: 342-349.
10. Meng ZQ, Yang PY, Shen YH, Bei WY, Zhang Y et al. (2009) Pilot study of huachansu in patients with hepatocellular carcinoma, nonsmall-cell lung cancer or pancreatic cancer. Cancer 115: 09-18.
11. Liu YF, Xiao YS, Xue XY, Zhang XL, Liang XM (2010) Systematic screening and characterization of novel bufadienolides from toad skin using ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry 24: 667-678.
12. Cao XT, Wang D, Wang N, Dai YH, Cui Z (2009) Isolation and identification of bufogenin constituents from the skin of Bufo bufo gargarizans Cantor. Journal of Shenyang Pharmaceutical University 26:778-781.
13. Wang YG, Gu SL (2009) RP-HPLC determination of cinobufotalin in Bufo gargarizans. Chinese Journal of Pharmaceutical Analysis 29: 1172-1174.
14. Ai YJ, Wang D, Dai YH, Cao XT, Li X, Cui Z (2009) Determination of the content of bufogenin in toad skin by HPLC. Journal of Shenyang Pharmaceutical University 26: 290-292.
15. Gao HM, Martin Z, Alexander L, Wu XY, Wang ZM et al. (2010) Comparison of toad venoms from different Bufo species by HPLC and LC-DAD-MS/MS. Journal of Ethnopharmacology 131: 368-376.
16. Ma XC, Zhang BJ, Xin XL, Huang SS, Deng S et al.(2009) Simultaneous quantification of seven major bufadienolides in three traditional Chinese

Components[a]	Content (μg g^{-1})										
	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	RSD (%)
gamabufotalin	409.7	1080.0	502.7	572.6	1862.3	1255.8	537.8	1163.7	297.1	554.0	60.25
arenobufagin	144.2	355.0	533.4	267.5	999.9	305.2	762.0	280.2	334.0	286.4	61.85
telocinobufagin	69.2	189.5	290.6	140.1	702.9	197.2	444.2	187.6	190.1	136.9	73.50
desacetylcinobufotalin	410.2	479.8	817.1	530.0	1680.9	271.6	1026.3	194.2	389.3	404.4	72.16
bufotalin	550.2	1015.1	817.3	814.1	2027.8	1084.6	960.7	1115.5	541.2	678.9	44.53
cinobufotalin	128.4	634.3	1009.8	428.8	2021.6	634.0	1577.9	566.4	670.4	487.8	70.19
bufalin	1619.3	1420.3	1025.8	1229.1	2926.8	1630.3	1076.5	1893.3	832.2	809.5	43.73
cinobufagin	710.3	2259.5	2011.3	1516.2	4850.1	2367.5	2548.3	2216.8	1255.3	1594.4	52.10
resibufogenin	2332.8	1050.5	311.4	1304.6	1927.5	1397.1	404.2	2030.4	761.0	258.4	63.74

Table 4: Sample analysis.

medicinal preparations of chansu by HPLC-DAD. Natural Products Communication 4: 179-184.

17. Ye M, Guo H, Guo HZ, Han J, Guo DA (2006) Simultaneous determination of cytotoxic bufadienolides in the Chinese medicine ChanSu by high-performance liquid chromatography coupled with photodiode array and mass spectrometry detections. Journal of Chromatography 838: 86-95.

18. Zhao DZ, Chen JY, Qin Y, Jiang YT (2006) A comparison study on the chemical constituents of Venenum Bufonis and the skin of toad. Tianjin Pharmacy 18: 21-24.

Citation: Wang SW, Duan LR, Cao W, Xie YH, Yuan JN, et al. (2015) Simultaneous Quantitative Determination of Nine Bufadienolides in Traditional Chinese Medicinal Toad Skin from Different Regions of China by High-Performance Liquid Chromatography–Photodiode Array Detection. Pharm Anal Acta 6: 345. doi:10.4172/2153-2435.1000345