Asian Journal of Chemistry Vol. 22, No. 8 (2010), 5883-5890

Comparative Estimation of (α + β) Boswellic Acid and Curcumin from Marketed Herbal Antirheumatic Tablets

M.D. WANDHARE*, U.A. DEOKATE, S.S. KHADABADI, S.P. HADKE and S.L. DEORE
Department of Pharmacognosy and Phytochemistry,
Government College of Pharmacy, Kathora naka, Amravati-440 064, India
E-mail: mangeshwandhare84@gmail.com

A rapid and sensitive high-performance thin-layer chromatographic (HPLC) method was developed and validated for the quantitative estimation of boswellic acids in formulation containing *Boswellia serrata* extract (BSE). Simple extraction method was used for isolation of boswellic acid from formulation sample. The isolated samples were chromatographed by using RP-HPLC method on HiQ Sil C_{18} W 4.6 mm × 250 mm i.d. column and detected by UV at 210 nm. The linearity range for (α + β) boswellic acid was100-500 mg^3/mL with average recovery of 98.14 ± 0.9362. The limit of detection and limit of quantification were found to be (0.01740, 0.05273, 0.01739 and 0.05270 mg^3/mL) for α and β boswellic acid, respectively. The developed method was successfully applied for the assay of market formulations containing *Boswellia serrata* extract. A rapid and sensitive high-performance thin-layer chromatographic (HPLC) method was developed and validated for the quantitative estimation of curcumin in formulation containing *Curcuma longa* extracts. Simple extraction method was used for isolation of curcumin from formulation sample. The isolated samples were chromatographed by using RP-HPLC method on HiQ Sil C_{18} W 4.6 mm × 250 mm i.d. column and detected by UV at 420 nm. The linearity range for curcumin was 0.4 -2.4 mg^3/mL with average recovery of 97.57 ± 0.5137. The limit of detection and limit of quantification was found to be 3.0196 and 9.1503 for curcumin, respectively. The developed method was successfully applied for the assay of market formulations containing *Curcuma longa* extracts.

Key Words: RP-HPLC, *Curcuma longa* extracts, Curcumin, *Boswellia serrata* extract, α and β boswellic acid.

INTRODUCTION

Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the Ayurvedic medicines is the lack of standard quality control profiles. The quality of herbal medicine *i.e*., the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of plant-based drugs, it is difficult to establish quality control parameters. To over come these problems modern analytical techniques are expected to help in circumventing this problem[1,2].

Boswellia serrata plant contains boswellic acid as their major active constituent which is present in *Boswellia serrata* extract as α-boswellic acid, β-boswellic acid (0.49 % w/w), 3-O-acetyl-11-keto β-boswellic acid (0.50 % w/w)[3], which are responsible for antirheumatic activity.

From ancient times *Boswellia serrata* is used for treating rheumatic arthritis disorders solely because of potency of these active constituents. Boswellic acid shows its activity by inhibiting synthesis of pro-inflammatory cytokines and 5- lipoxygenase activity[4]. Nowadays *Boswellia serrata* extract is used in most marketed formulations.

Rhizomes of *Curcuma longa* contains curcuminoids (50-60 %) which contains curcumin (*ca.* 6 %)[5] as active constituent responsible for antirheumatic activity. From the ancient times it is known and used more efffectively for antiinflammatory and antirheumatic activity. Curcumin shows antirheumatic activity by inhibiting both 5-lipoxygenase and 12-lipoxygenase activity[4], also it inhibits the NF-κB and promotes adhesion of neutrophils to human umblical veins endothelial cells[6]. Nowadays *Curcuma longa* extract is used in most marketed formulations. Also many phytoconstituents are analyzed by HPLC due to its versatility, safety, dependability and sensitivity. Hence HPLC is being used in the laboratories of many pharmaceutical companies for the routine assay of new drugs, as well as substitutes for older, more troublesome assays for marketed drugs. So, estimation of curcumin in those formulations by using HPLC chromatographic techniques paves major role[7].

EXPERIMENTAL

All the formulations were procured from local market of Amravati. All the chemicals used in formulatins were of analytical grade. Standard (α + β) boswellic acid (purity 97 %) and curcumin (purity 99 %) purchased from Natural Remedies Pvt. Ltd., Bangalore. All the solvents used in experiments were of analytical grade.

Preparation of standard solutions of (α + β) boswellic acid: The standard solution are prepared by dissolving 5 mg of boswellic acid in 2.5 mL methanol (HPLC grade) and sonicate for 15 min and used as a stock solution. (2000 µg/mL). Working standard solution are prepared by pippeting 0.05, 0.1, 0.15, 0.20 and 0.25 mL and by making volume with mobile phase so that final concentration of 100, 200, 300, 400 and 500 mg^3/mL is achieved which is then sonicated for about 15 min and then used as working standard.

Preparation of standard solutions of curcumin: The standard solution are prepared by dissolving 5 mg of curcumin in 2.5 mL of methanol (HPLC grade) and sonicate for 15 min and used as a stock solution (2000 µg/mL). Working standard solution are prepared by pippeting 0.1 mL of stock solution to 10 mL which produce 20 µg/mL by making volume with mobile phase from this solution 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL were pipetted and volume was made by mobile phase to obtain concentration of 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 µg/mL is achieved which is then sonicated for about 15 min and then used as a working standard.

Preparation of sample solutions

Determination of boswellic acid (α + β) in formulation No. 1 (batch No. 1 and 2): Sample preparation is done to evaluate active constituent present in the extracts of resin. The amount of β-boswellic acid reported in resin extract is 0.49 % w/w. So weight of 4 tablets was taken approximately corresponding to 2680 mg (avg wt is 693.3 and 691.5 mg, respectively for both the batches, respectively) and powdered in mortar and pastel to obtain a powdery mass which is then extracted with diethyl ether for several times until a clear solvent is obtained at last giving indication that complete extraction is achieved the solvent then evaporated and the final solution is prepared upto 5 mL by adding mobile phase with filtration. Sonication is done for 15 min before injecting sample in injection port.

Determination of boswellic acid (α + β) in formulation No. 2 (batch No. 1 and 2): Sample preparation is done to evaluate active constituent present in the extracts of resin. The amount of β-boswellic acid reported in resin extract is 0.49 % w/w. So weight of 1 tablet was taken approximately corresponding to 1125 mg (avg wt is 1183.3 and 1170.8 mg, respectively for both the batches, respectively) and powdered in mortar and pastel to obtain a powdery mass and 675 mg of powder which is then extracted with diethyl ether for several times until a clear solvent is obtained at last giving indication that complete extraction is achieved the solvent then evaporated and the final solution is prepared upto 10 mL by adding mobile phase with filtration. Sonication is done for 15 min before injecting sample in injection port.

Determination of curcumin in formulation No. 1 (batch No. 1 and 2): Sample preparation is done to evaluate active constituent present in the extracts of rhizome. The amount of cucumin reported in rhizome extract is *ca.* 6 % w/w. So weight of 1 tablet was taken approximately corresponding to 675 mg and powdered in mortar and pastel to obtain a powdery mass and 38 mg of powder which is then dissolved in mobile phase and final solution was made upto 10 mL by filtration (2 µg/mL).

Determination of curcmin in formulation No. 2 (batch No. 1 and 2): Sample preparation is done to evaluate active constituent present in the extracts of rhizome. The amount of β-boswellic acid reported in resin extract is *ca.* 6 % w/w. So weight of 1 tablet was taken approximately corresponding to 1125 mg (avg wt is 1183.3 and 1170.8 mg, respectively for both the batches, respectively) and powdered in mortar and pastel to obtain a powdery mass which is then dissolved in mobile phase and final solution was made upto 10 mL by filtration (2 µg/mL).

Calibration curve for (α + β) boswellic acid and curcumin: 20 µL of standard solution of (α + β) boswellic acid and curcumin was injected in injection port of HPLC system by using mobile phase methanol: 5 % acetonitrile solution in water buffered to pH 2.7 by 10 % ortho-phosphoric acid (90:10 v/v) on HiQ Sil C_{18} W size, 4.6 mm × 250 mm i.d. column with a flow rate of 2 mL/min at a pressure of 18-20 and 16-20 mpascal at ambient temperature. The peak was detected at 210 and 420 nm. The peak areas were recorded and calibration curve was prepared by plotting peak areas *versus* concentration applied.

Quantification of (α + β) boswellic acid and curcumin: 20 µL of sample solution of (α + β) boswellic acid and curcumin was injected in injection port of HPLC system by using mobile phase methanol: 5 % acetonitrile solution in water buffered to pH 2.7 by 10 % ortho-phosphoric acid (90:10 v/v) on HiQ Sil C_{18} W size, 4.6 mm × 250 mm i.d. column with a flow rate of 2.0 and 1.5 mL/min at a pressure of 16-20 mpascal at ambient temperature. The peak was detected at 210 and 420 nm. Peak areas and absorption chromatogram were recorded. To check the identity, peak chromatogram of standard was overlayed with the corresponding chromatogram of sample solution. The amount of curcumin in the sample was calculated.

The amount of each drugs estimated in laboratory mixture was calculated by using following formula.

$$E_w = \frac{A_u}{A_s} \times C_s \times d$$

where, E_w = drug estimated in sample weight (mg), C_s = concentration of standard (µg/mL), A_u = area of unknown, A_s = area of standard, d = dilution factor.

The results indicate that the laboratory mixture containing (α + β) boswellic acid and curcumin can be estimated accurately by proposed method; so it was thought to apply the same procedure for marketed formulation.

Amount of drug present in average weight of tablet as per of labeled claim was calculated using following formula:

$$\text{Labeled claim}\,(\%) = \frac{E_w \times W_{AV}}{L_c \times W_s} \times 100$$

where, E_w = amount estimated (mg), W_{AV} = average weight of tablet (mg), W_s = sample weight (mg), L_c = labeled claim (mg/tablet).

Validation of method

For (α + β) boswellic acid: The method was validated for precision, repeatability and accuracy. The method repeatability was carried out by injecting five replicates of sample solution (200 mg^3/mL). Variability of the results was studied by injecting sample solution on same day (intra day), on different days (inter day) and by different analyst.

Accuracy of the method was tested by performing recovery studies at three levels (80, 100 and 120 % addition). The per cent recovery as well as average per cent recovery was calculated. Limit of detection and limit of quantitation was evaluated by slope and standard deviation of the response.

For curcumin: The method was validated for precision, repeatability and accuracy. The method repeatability was carried out by injecting five replicates of sample solution (2 mg^3/mL). Variability of the results was studied by injecting sample solution on same day (intra day), on different days (inter day) and by different analyst.

Accuracy of the method was tested by performing recovery studies at three levels (80, 100 and 120 % addition). The per cent recovery as well as average per cent recovery was calculated. Limit of detection and limit of quantitation was evaluated by slope and standard deviation of the response.

RESULTS AND DISCUSSION

For (α + β) boswellic acid: Formulations 1 and 2 contains *Boswellia serrata* extracts which contains α and β boswellic acid as major actives. For quantification of this marker from these formulations HPLC method was developed. Mobile phases were optimized and extraction was carried out by using petroleum ether solvent for better resolution of marker compound from the other components of the sample extracts. Of the various mobile phases tried ,the one containing mobile phase methanol: 5 % acetonitrile solution in water buffered to pH 2.7 by 10 % ortho-phosphoric acid (90:10 v/v) gave best resolution at retention time 14.9 and 16.9 for α and β boswellic acid, respectively in the presence of other compounds in the sample extract and enabled the quantification of the marker compounds. To check the identity and purity, peak chromatogram of standard was overlayed with the corresponding chroma-togram of sample solution. The method was validated in terms of precision, repeatability and accuracy studies (Table-1).

TABLE-1
RESULTS OF METHOD AND INTERMEDIATE PRECISION (α + β) BOSWELLIC ACID

Formulations	Parameters (%)	Method repeatability	Intermediate precision		
			Interday	Intraday	Different analyst
Formulation 1 batch No. 1	Percentage estimation	80.84 ± 0.1259	80.47 ± 0.0565	80.46 ± 0.040	80.63 ± 0.1157
	RSD	0.1557	0.0700	0.049	0.1435
Formulation 1 batch No. 2	Percentage estimation	72.45 ± 0.06744	72.30 ± 0.0852	72.27 ± 0.0163	72.20 ± 0.0565
	RSD	0.093	0.1179	0.02259	0.0782
Formulation 2 batch No. 2	Percentage estimation	76.74 ± 0.062	76.70 ± 0.0532	76.65 ± 0.0556	76.61 ± 0.1059
	RSD	0.0818	0.0693	0.0726	0.1383
Formulation 2 batch No. 2	Percentage estimation	88.25 ± 0.2945	87.75 ± 0.0816	87.73 ± 0.0374	87.75 ± 0.0412
	RSD	0.3377	0.09299	0.0426	0.04695

*Mean ± SD (n = 3).

The relationship between the concentrations of standard solutions and peak response was linear within the concentration range of 100-500 mg^3/mL with a correlation coefficient of 0.9949 and 0.9955 for α and β boswellic acid, respectively. The average per cent recovery at three different levels was found and results are presented in Table-2. The content of α and β boswellic acid in the formulations was estimated by proposed method (Table-3). The content of α and β boswellic acid in

formulation 1 of batch No. 1 is more than batch No. 2 and in formulation 2 of batch No. 2 is more than batch No. 1. The content in all these batches of formulations is fall well behind the labeled claim. The method was found to be suitable for estimation of marker compounds from herbal tablets.

TABLE-2
RESULTS OF RECOVERY STUDY (α + β) BOSWELLIC ACID

Formulations	Level (%)	Added conc. (µg)	Recovered (µg)	Recovery (%)	Mean ± SD	RSD (%)
Formulation 1 batch No. 1	80	160	158	98.750	98.22 ± 0.4611	0.4695
	100	200	196	98.000		
	120	240	235	97.910		
Formulation 1 batch No. 2	80	160	154	96.250	97.13 ± 1.7567	1.8084
	100	200	192	96.000		
	120	240	238	99.160		
Formulation 2 batch No. 1	80	160	158	98.750	97.86 ± 1.7624	1.8010
	100	200	198	99.000		
	120	240	230	95.830		
Formulation 2 batch No. 2	80	160	159	99.375	98.40 ± 0.9395	0.9548
	100	200	195	97.500		
	120	240	236	98.330		
Average % recovery					98.14 ± 0.9362	0.9539

*Mean ± SD (n = 3).

TABLE-3
ESTIMATION OF (α + β) BOSWELLIC ACID FROM THE FORMULATIONS 1 AND 2

Formulations	(α + β) Boswellic acid w/w (%)
Formulation 1 batch No. 1	80.65 ± 0.1679
Formulation 1 batch No. 2	72.25 ± 0.0890
Formulation 2 batch No. 1	76.70 ± 0.1737
Formulation 2 batch No. 2	87.75 ± 0.1720

*Mean ± SD (n = 3).

For curcumin: Formulations 1 and 2 contains *Curcuma longa* extracts which contains curcumin as major actives. For quantification of this marker from these formulations HPLC method was developed. Mobile phases were optimized and extraction was carried out by using petroleum ether solvent for better resolution of marker compound from the other components of the sample extracts. Of the various mobile phases tried, the one containing mobile phase methanol: 5 % acetonitrile solution in water buffered to pH 2.7 by 10 % ortho-phosphoric acid (90:10 v/v) gave best resolution at retention time 2.333 for curcumin, respectively in the presence of other compounds in the sample extract and enabled the quantification of the marker compounds. To check the identity and purity, peak chromatogram of standard was overlayed with the corresponding chromatogram of sample solution. The method was validated in terms of precision, repeatability and accuracy studies (Table-4).

TABLE-4
RESULTS OF METHOD AND INTERMEDIATE PRECISION FOR CURCUMIN

Formulations	Parameters	Method repeatability mean ± SD	Intermediate precision		
			Interday	Intraday	Different analyst
Formulation 1 batch No. 1	Estimation	98.13 ± 0.174	98.47 ± 0.4417	98.56 ± 0.1533	98.83 ± 0.2149
	RSD	0.177	0.4485	0.1556	0.2175
Formulation 1 batch No. 2	Estimation	98.69 ± 0.1067	98.89 ± 0.2073	99.21 ± 0.1140	99.31 ± 0.1479
	RSD	0.1081	0.2096	0.1149	0.1489
Formulation 2 batch No. 2	Estimation	99.89 ± 0.15	99.64 ± 0.1774	99.59 ± 0.1398	99.72 ± 0.1271
	RSD	0.1501	0.1762	0.1404	0.1275
Formulation 2 batch No. 2	Estimation	101.41 ± 0.4878	100.68 ± 0.1387	100.27 ± 0.08786	100.62 ± 0.1447
	RSD	0.48	0.1392	0.08762	0.1438

*Mean ± SD (n = 3).

The relationship between the concentrations of standard solutions and peak response was linear within the concentration range of 0.4 -2.4 mg^3/mL with a correlation coefficient of 0.9926 for curcumin, respectively. The average per cent recovery at three different levels was found and results are presented in Table-5. The content of curcumin in the formulations was estimated by proposed method (Table-6). The content of curcumin in all these batches of formulations is found as per the labeled claim. The method was found to be suitable for estimation of marker compounds from herbal tablets.

TABLE-5
RESULTS OF RECOVERY STUDY FOR CURCUMIN

Formulations	Level (%)	Added conc. (µg)	Recovered (µg)	Recovery (%)	Mean ± SD	RSD (%)
Formulation 1 batch No. 1	80	1.6	1.58	98.75	97.22 ± 1.9679	2.0241
	100	2.0	1.9	95.00		
	120	2.4	2.35	97.91		
Formulation 1 batch No. 2	80	1.6	1.54	96.25	97.36 ± 1.0470	1.0754
	100	2.0	1.95	97.50		
	120	2.4	2.36	98.33		
Formulation 2 batch No. 1	80	1.6	1.55	96.87	98.34 ± 1.2784	1.2999
	100	2.0	1.98	99.00		
	120	2.4	2.38	99.16		
Formulation 2 batch No. 2	80	1.6	1.56	97.50	97.39 ± 0.6717	0.6897
	100	2.0	1.96	98.00		
	120	2.4	2.32	96.67		
Average recovery (%)					97.57 ± 0.5137	0.5264

*Mean ± SD (n = 3).

TABLE-6
ESTIMATION OF CURCUMIN FROM THE FORMULATIONS 1 AND 2

Formulations	Curcumin (w/w) (%)
Formulation 1 batch No. 1	99.70 ± 0.1529
Formulation 1 batch No. 2	100.85 ± 0.4882
Formulation 2 batch No. 1	98.50 ± 0.1024
Formulation 2 batch No. 2	98.70 ± 0.1067

*Mean ± SD (n = 3).

Conclusion

We established HPLC method for the quantification of (α + β) boswellic acid and curcumin from the formulations. The method was found to be simple, precise, specific, sensitive and accurate and can be used for their quantification in the plant materials and also in routine quality control of the raw materials as well as formulations containing any or all of these compounds.

REFERENCES

1. L.V. Asokar, K.K. Kakkar and O.J. Chakra, Glossary of Indian Medicinal Plants with Active Principles, (Publication and Information Directorate, New Delhi, India, p. 122 (1992).
2. M.N. Ravishankara, N. Shrivastava, H. Padh and M. Rajani, *Planta Med.*, **67**, 294 (2001).
3. H.P. Ammon, H. Safayhi, T. Mack and J. Sabieraj, *J. Ethnopharmacol.*, **38**, 113 (1993).
4. V.D. Rangari, Textbook of Pharmacognosy Part II, Career Publication, Nashik, India, p. 354 (2005).
5. Indian Herbal Pharmacopoeia, Revised new edition, pp. 169-176 (2002).
6. B. Madan, W.N. Gade and B. Ghosh, *J. Ethnopharmacol.*, **75**, 25 (2001).
7. S.B. Gokhale, C.K. Kokate and A.P. Purohit, Textbook of Pharmacognosy, edn. 1, pp. 118-119 (2002).

(*Received*: 7 August 2009; *Accepted*: 28 April 2010) AJC-8637