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NOTE

Simultaneous Estimation of Piperine and 6-Gingerol in a Mixture of *Piper nigrum* and *Gingiber officinale* Through HPLC

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A rapid and sensitive HPLC method has been developed for the estimation of piperine and 6-gingerol in a mixture of *Piper nigrum* and *Gingiber officinale*. The analytes were separated from the mixture by using a suitable solvent. A Kromasil C_{18} column provided chromatographic separation of the analytes which was followed by detection at 280 nm. This method involves extraction of analytes through simple HPLC condition with mobile phase (methanol:water) and detection through UV detector. The retention times were 05.00 and 03.27 min for piperine and 6-gingerol, respectively. The proposed method can be used for the qualitative as well as quantitative determination of the piperine and 6-gingerol in various Ayurvedic preparations such as churnas and tablets.

Key Words: HPLC, Piperine, 6-Gingerol, *Piper nigrum*, *Gingiber officinale*.

Piper nigrum (commonly known as black pepper) belongs to Piperaceae family. It is a trailing or climbing shrub with fruits that are glabrous, nearly 6 mm in diameter, initially green and turning black on drying. The pungent principle in pepper fruit is an alkaloid analog compound, piperine; an amide of 5-(2,4-dioxymethylene-phenyl)-hexa-2,4-dienoic acid (piperinic acid)[1-4].

Zingiber officinale (commonly known as sunthi) belongs to Zingiberaceae family. The large flesh rhizome ginger root, although it is not a root, is the part used. It has a characteristic staghom-like appearance. The pungency of ginger is caused by the presence of Zingerone, gingeroles and shoagoles. The pungent gingeroles degrade to the milder Shoagoles during storage; high gingerole content and good pungency thus indicate freshness and quality[5-7].

Piper nigrum and *Zingiber officinale* have a long history of use in combination in various Ayurvedic formulation[8,9] such as Talisadi churna, Sitopaladi churna, *etc*.

All chemicals and reagents used were of HPLC grade. The HPLC grade methanol and water were obtained from Qualigens Fine Chemicals, Mumbai. The standards 6-gingerol and piperine were procured from Acros Organics (Belgium). The Churna containing both *Piper nigrum* and *Zingiber officinale* manufactured by Dabur India Ltd. was obtained from local market.

Standard solutions: The standard solution of piperine and 6-gingerol were prepared in methanol at free base concentration of 1000 µg/mL. Precautions were taken during the preparation of piperine solution to protect it from light (to prevent its isomerization) by using amber coloured glasswares. Mobile phase, methanol:water was used in different composition at different rate (1.0, 1.2, 1.5, 1.8 mL/min). The composition of the mobile phase methanol:water in the ratio of 30:70 % v/v at flow rate of 1.5 mL/min gave sharp peaks with minimum tailing and good resolution for piperine and 6-gingerol, whereas no such good resolution was obtained from other combinations. Table-1 shows the optimized chromatographic conditions.

TABLE-1
OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Parameters method	
Stationary phase (column)	Kromosil C_{18} Symmetry (4.6 × 250 mm)
Mobile phase	Methanol:water (70:30)
Flow rate (mL/min)	1.5
Column temperature (°C)	Ambient
Volume of injection (µL)	10
ISTD conc.	1.000
Polarity	Positive
Std. 1 retention time (min)	05:000
Std. 2 retention time (min)	0.3:000
Run time	15min

Sample solution: 50 mg of the powdered drug was refluxed with 100 mL of methanol for 0.5 h. The resulting

solution was filtered and the marc was further refluxed with 100 mL of methanol. The combined filtrate was then evaporated under vacuum to about 25 mL. The resulting solution was cooled to room temperature. The volume was made up to 100 mL and suitable dilution was prepared.

Procedure: The known volumes (10 µL) of the Std. 1, Std. 2 and the sample prepared were injected into the HPLC and the retention time for the standards and sample were observed.

The HPLC chromatogram for the standard 1 (Fig. 1) shows the retention time of piperine at 0.5:00 mm:ss. The HPLC chromatogram for the standard 2 (Fig. 2) shows the retention time of 6-gingerol at 03:27 mm:ss. The HPLC chromatogram for the churna, containing both piperine (in the form of *Piper nigrum*) and 6-gingerol (in the form of *Zingiber officinale*) shows the peak (Fig. 3) for both the constituents exactly at the same retention time (03:27 mm:ss and 04:59:6 mm:ss) which proves the presence of both the constituents in the churna.

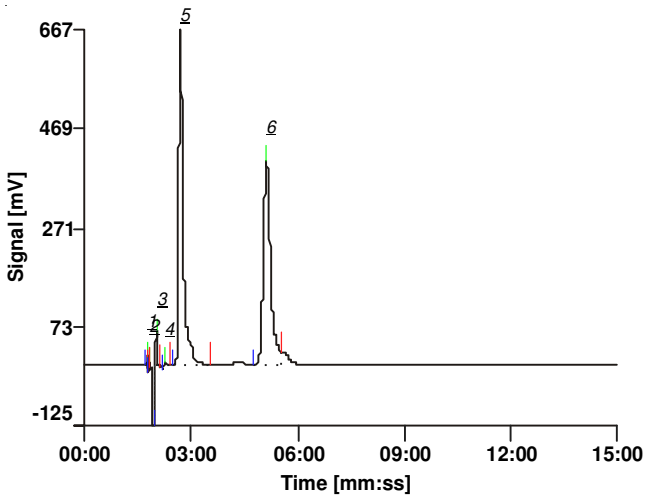

Fig. 1. HPLC chromatogram for piperine (Std. 1)

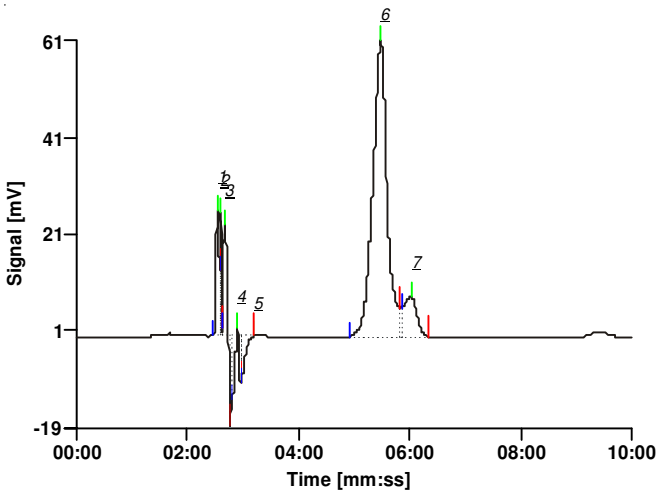

Fig. 2. HPLC chromatogram for 6-gingerol (Std. 2)

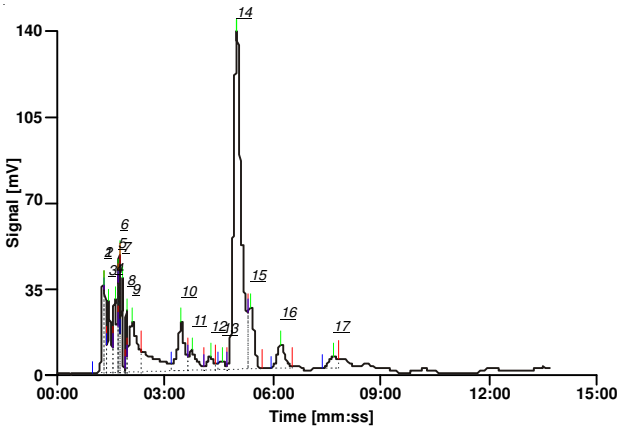

Fig. 3. HPLC chromatogram for churna (sample)

Conclusion

The HPLC method was found to be good for the simultaneous estimation of piperine and 6-gingerol in a mixture; it can be suitable for the application of routine quality control analysis as well as may be further extended to the quantitative determination of the above components in any ayurvedic mixture.

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REFERENCES

1. K. Sivaraman, K. Kandiannan, K.V. Peter and C.K. Thankamani, *J. Spices Aromat. Crops*, **8**, 1 (1999).
2. T. Thangaselvabal, C.G.L. Justin and M. Leelamathi, *Agricult. Rev.*, **29** (2008).
3. Indian Herbal Pharmacopoeia, A Joint Publication of Regional Research Laboratory, Jammu Tawi and Indian Drug Manufacturer's Association, Mumbai, Vol. 2, p. 93 (1999).
4. F.N. McNamara, A. Randall and M.J. Gunthorpe, *Br. J. Pharmacol.*, **144**, 781 (2005).
5. J.S. Kim, S.I. Lee, H.W. Park, J.H. Yang, T.Y. Shin and Y.C. Kim, *Arch. Pharmacol. Res.*, **31**, 415 (2008).
6. Indian Herbal Pharmacopoeia, A Joint Publication of Regional Research Laboratory, Jammu Tawi and Indian Drug Manufacturer's Association, Mumbai, Vol. 2, p.162 (1999).
7. I.-M. Chung, M. Ali and A. Ahmad, *Asian J. Chem*., **17**, 1915 (2005).
8. D. Sobhana, A. Abhilasha, W. Cyril and B.S. Kumar, *Asian J. Chem*., **23**, 885 (2011).
9. The Ayurvedic Pharmacopoeia of India, Part I, 1, p.101.