Journal of Advanced Pharmaceutical Technology & Research

A SIMPLE AND RAPID METHOD FOR SIMULTANEOUS ESTIMATION OF GLYCYRRHETINIC ACID AND PIPERINE BY HPTLC IN A HERBOMINERAL FORMULATION

Ashish Trivedi*, S. H. Mishra

Herbal Drug Technology, Pharmacy Department, Faculty of Technology and Engineering, The M. S. University of Baroda, Kalabhavan, Vadodara, Gujarat (India)

Corresponding Author's E-mail: - ajt402@gmail.com

Received: 03rd April 2010 Revised: 29th May 2010 Accepted: 06th June 2010

ABSTRACT

Many of the traditional herbal formulations contain extracts of *Piper longum* and *Glycyrrhiza glabra*, piperine and glycyrrhetinic acid respectively, being active constituents of these two herbs. An attempt has been made to develop a simple, precise, rapid, and cost-effective high-performance thin-layer chromatographic (HPTLC) method for simultaneous estimation of these in a herbomineral formulation (Efiplus® Capsules). Precoated silica gel 60 F_{254} plates with toluene-ethyl acetate-glacial acetic acid 12.5:7.5:0.5, as mobile phase were used in chromatographic determinations. The plates were scanned and the compounds were quantified at their wavelengths of maximum absorption of 260 and 331 nm for glycyrrhetinic acid and piperine respectively. The respective R_F values of glycyrrhetinic acid and piperine were 0.51 and 0.55. Under these experimental conditions linearity was observed between 0.8-2.6 µg/spot for glycyrrhetinic acid and between 10-50 ng/spot for piperine and average recovery was 96.25% for glycyrrhetinic acid and 98.55% for piperine.

Key words: HPTLC, glycyrrhetinic acid, piperine, herbomineral formulation.

INTRODUCTION

Herbal medicines are the oldest remedies known to mankind; these generally contain more than one herb in the combination. Although, considerable work is being done to evaluate herbals for their quality, safety and efficacy, still a need of, a well-defined specific method for routine analysis of herbal raw materials and formulations with regard to constituents that may be responsible for efficacy is generally felt. Development of methods for analysis of plant products poses difficulty, due to their unknown chemical profile, more so in case of multi components herbal formulations.

In the present study, an attempt has been made to develop a simple, rapid and accurate HPTLC method for

simultaneous estimation of glycyrrhetinic acid and piperine in a marketed herbomineral capsule formulation (Efiplus® Capsules) indicated for use in iron deficient anaemia.

Glycyrrhetinic acid is one of the main active constituent of roots of *Glycyrrhiza glabra*. It is a recognized medicine in India as an expectorant traditionally and used in various preparations affecting gastrointestinal system. The herb also contains glycyrrhizin, flavonoids, isoflavonoids, and chalcones as major active chemical constituents [1]. The herb is reported with the following studies antiulcer & antioxidant, antioxidant and wound healing, anxiolytic, carminative and antiemetic, antifungal, nephroprotective, antimicrobial, immuno-modulatory, antiinflammatory, antiasthmatic [2-3]. Pippali (*Piper longum*) is a powerful stimulant for both the digestive and the respiratory systems [4]. Pippali a typical ayurvedic complementary component whose benefit is to increase the bioavailability and enhance absorption of the other active ingredients [5-6]. It contains mainly alkaloids (piperine as major alkaloid) and amides, lignins, esters, volatile oils. The whole plant is considered by tribal people in India to be useful in splenic disorders, cholera, dysentery, asthma, cough and bronchitis [7-8]. It is studied for various biological activities viz. immunomodulatory activity, stimulant activity, antiasthmatic activity, hepatoprotective activity, hypocholesterolaemic activity, antiinflammatory activity [9-11].

These two herbs are most common components of ayurvedic medicine and herbal medicines used for treating gastrointestinal, respiratory system and metabolic disorders. Some of the marketed ayurvedic medicines that contain these two herbs are Khadirarishta, Lohasava, Ashvagandharishta, Dasmularishta, Chandanasava, Satvaryadi ghrita etc. Various herbal medicines used as antacid, hepatoprotective, antiulcer, dyspeptic, haematinic preparations etc are also known to contain these two herbs e.g. Deepana capsule, Dicolai capsule, Suryacid tablet, Diobliv, panchasav, efiplus caps, neotab etc.

MATERIALS AND METHODS

All the solvents and reagents of analytical grade purchased from M/s. Qualigens India Ltd. were used. The solvents were redistilled before use. Glycyrrhetinic acid and piperine standards were purchased from sigma chemicals. Precoated silica gel plates (TLC plates, silica gel aluminium sheets with 60 F_{254}) were purchased from M/s. Merck India Ltd.

1) Herbomineral formulation

Selected marketed herbomineral capsule formulation (constituents are given in

table 1), indicated for use in iron deficient anaemia, contains *Shuddha kasis* (a mineral component- ferrous sulphate), powdered herbs; *Cyprus rotundus*, *Piper longum*, *Zingiber officinale* and aqueous extract of *Glycyrrhiza glabra*. *Shuddha kasis* is the source of iron and the other herbs act as bioavailability enhancer, antioxidant and reduce nausea-vomiting.

Table 1: Constituents of selected marketed herbomineral formulation (Efiplus® Capsules)

Herbs added	Part used	Quantity used
Shuddha kasis	mineral	200 mg
Embelica officinalis	Fruits powder	100 mg
Zingiber officinale	rhizome powder	100 mg
Cyperus rotundus	rhizome powder	100mg
Piper longum	Fruits powder	100 mg
Glycyrrhiza glabra	water extract eq. to	100 mg

2) HPTLC instrument

The samples were spotted in the form of bands with 8 mm size on precoated Silica gel plates using Linomet V (Camag) applicator. The plates were washed with methanol and activated at 120^{0}C for 10 min. prior to application. The application rate was set at 150 nL/ sec. The monochromator band width was set 20 nm, each track was scanned thrice and base line correction was used. Mobile phase toluene: ethyl acetate: gl.acetic acid (12.5:7.5:0.5), was used for development of sample. The plates were allowed to dry at room temperature (25 ± 2^0) at relative humidity of 60% ±5. The optimized chamber saturation time was 35 min. at room temperature (25 ± 2^0).

The dried plates were scanned and quantified in reflectance- absorbance mode at 260 nm and 331 nm using the CAMAG TLC SCANNER-3.

A) Calibration curve for glycyrrhetinic acid and piperine

A stock solution of glycyrrhetinic acid (220 µgmL^{-1}) and piperine (5 µgmL^{-1}) was prepared in methanol. Aliquot of above solution (2, 4, 6,8,10 and 12 µL) was applied with the band width of 8 mm, on TLC plate (10X20 cm) silica gel 60 F254 Merck.

The plate was developed as above procedure. After developing the plates were dried and standard calibration curves of piperine and glycyrrhetinic acid were plotted at 331 nm and 260nm respectively using TLC SCANNER-3 (CAMAG). Peak areas for each band were recorded. Calibration curve was obtained by plotting peak area vs concentration of glycyrrhetinic acid and piperine.

B) Preparation of Test sample

Test sample (1.5 mgmL^{-1}) was prepared by acid hydrolysis. Sample was taken from 20 capsules and 100 mL of 1N HCl solution was added in a round bottom

flask. This mixture was refluxed for an hour and cooled and filtered. Filtrate is taken in a separating funnel and extracted with chloroform till free of alkaloid. Chloroform extract is dried in vacuum and residue was dissolved in methanol to get concentration of 1.5 $mgmL^{-1}$. 10 µL of sample solution was applied on same plate in triplicate and the plate was developed as above procedure. After developing the plate was dried and peak areas for each band were recorded. Spectrum of piperine and glycyrrhetinic acid in test sample was confirmed by overlaying the spectra of standard calibration curve.

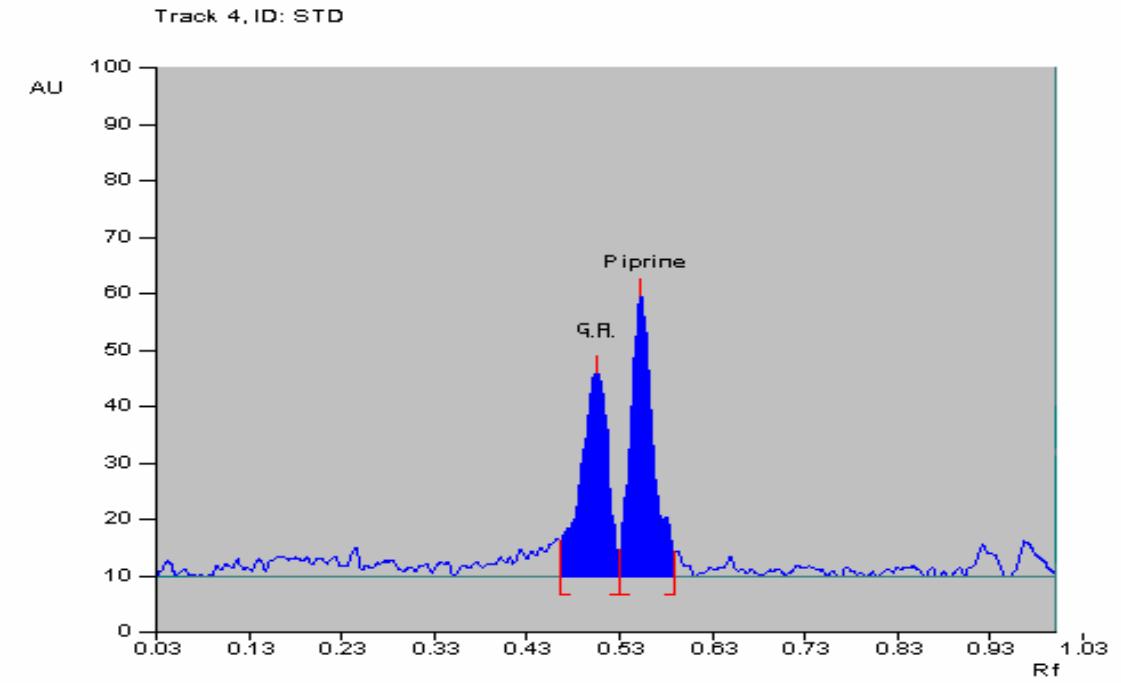

Fig. 1: Peaks showing glycyrrhetinic acid and piperine in standard solution at 254 nm

3) Method validation

The developed method was validated in terms of Linearity, accuracy and precision according to ICH guidelines [12-13].

A) Accuracy

The recovery studies were performed by applying the known amount of the samples and the percentage recovery of that same amount is been calculated against the theoretical values. Pre-analyzed samples were applied at three different concentration levels of the standard (80%, 100% and 120% w/w) containing piperine and glycyrrhetinic acid and analyzed with the instrument set up as same in case of the estimation of the sample. This was done to check the recovery of the drug at different

levels in the formulations. The experiments were performed in triplicate.

B) Repeatability

Repeatability (precision) was determined by repeated analysis of standard sample using the same equipment, same analytical procedures, and same laboratory and on the same plate. Repeatability of measurement was determined by spotting 10 µL of standard drug solution on TLC plate, after development spot was scanned six times without changing position. The % RSD was determined for piperine and glycyrrhetinic acid.

C) Linearity and specificity

Linearity was determined by spotting various concentrations of standard and finding regression. The specificity of the method was ascertained by comparing the R_f value and the peak purity was assessed by comparing the spectrum of piperine and glycyrrhetinic acid with those acquired at the peak start, peak apex, and peak end positions of sample bands.

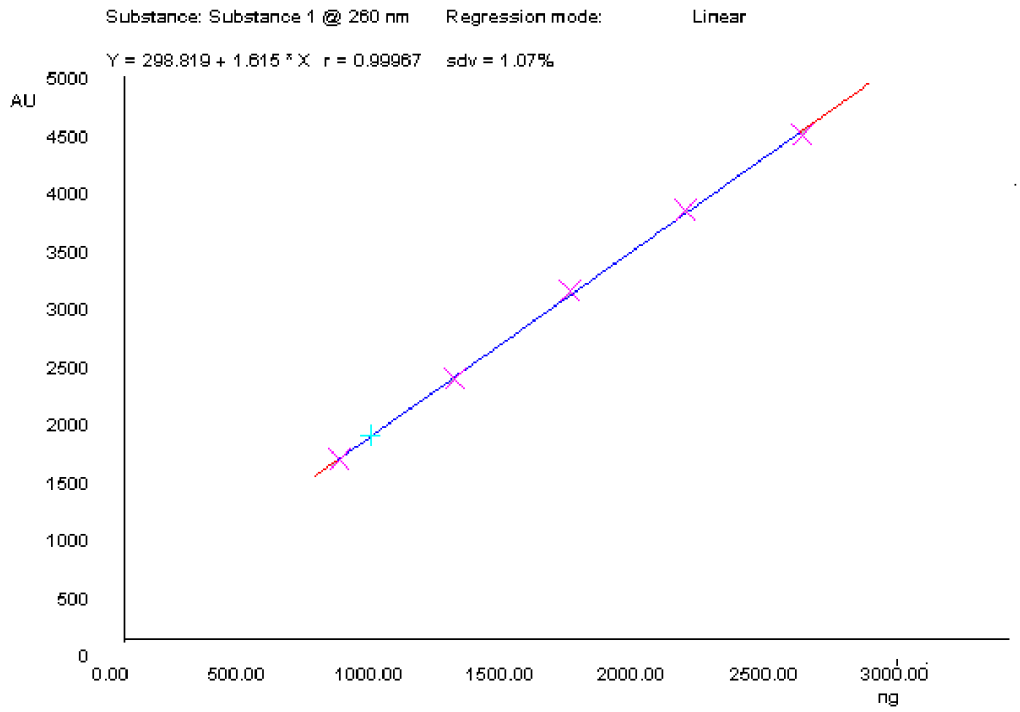

Fig. 2: Calibration curve of glycyrrhetinic acid

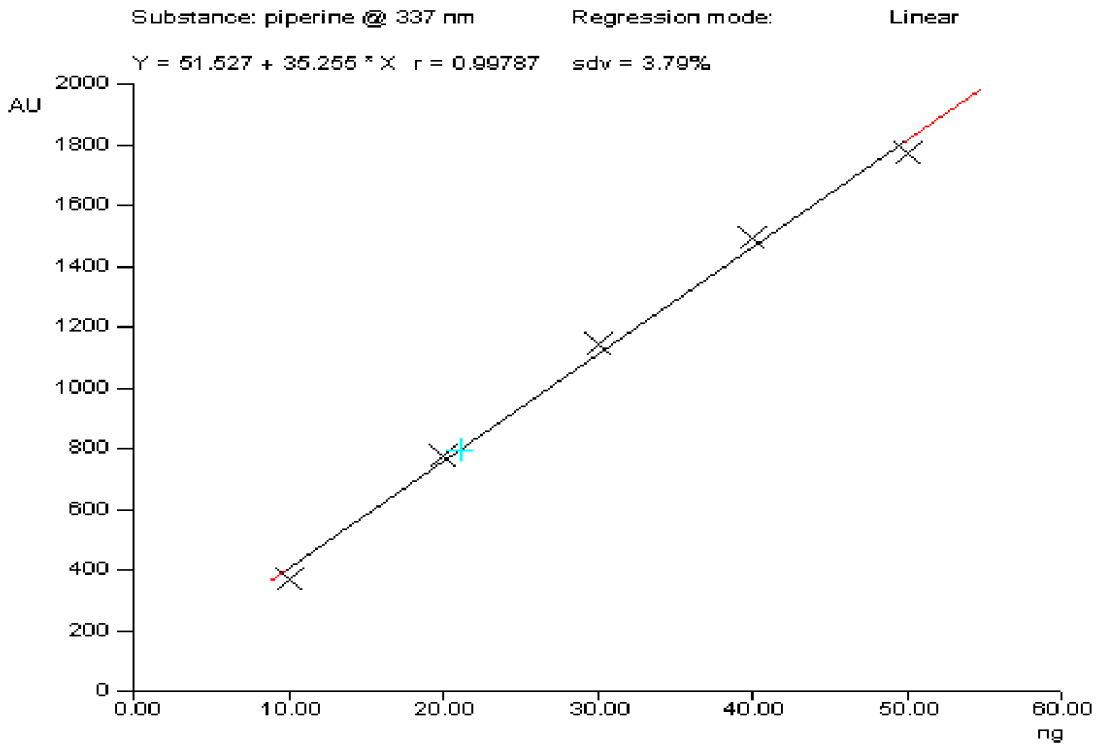

Fig.3: Calibration curve of piperine

RESULTS AND DISCUSSIONS

Optimization of mobile phase

Various proportions of toluene, ethyl acetate, acetone, chloroform were tried. The mobile phase containing Toluene: ethyl acetate: gl.acetic acid (12.5:7.5:0.5), gave sharp and symmetric peaks and improved spot characteristics for glycyrrhetinic acid and piperine both. The first spot at R_f 0.51 was identified as glycyrrhetinic acid and the second spot at R_f 0.55 was identified as piperine with the help of the chromatograms of their individual standards.

Calibration curves of standard glycyrrhetinic acid and piperine

Calibration graph was found to be linear and was evaluated by determining six standard spots containing 0.8-2.6 µg/spot for glycyrrhetinic acid and between 10-60 ng/spot for piperine respectively. Peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients.

Method Validation

The regression data as shown in Table 2 describe a good linear relationship over concentrations 0.8-2.6 µg/spot for glycyrrhetinic acid and between 10-60

ng/spot for piperine with the r^2 = 0.9997and 0.9978 respectively. Average recovery was 96.25% for glycyrrhetinic acid and 98.55% for piperine as shown in Table 3. The % RSD was found to be 0.3639 and 1.0391 for piperine and glycyrrhetinic acid respectively for repeatability of the method. The results shown meet the acceptance criterion for % RSD specified by the ICH [12-13] which is a precision of less than 2-3 % RSD.

Table 2: Method validation parameters for glycyrrhetinic acid and piperine by HPTLC

Parameters	Range	
	Glycyrrhetinic acid	**Piperine**
Linearity range	0.8-2.6 µg/spot	10 – 50 ng/spot
Correlation coefficient (r)	0.9997	0.9978
Regression equation	Y = 298.81 + 1.615*X	Y = 51.527 + 35.25* X
Slope (m)	1.615	35.255
Intercept (c)	298.81	51.527
Limit of detection (LOD)	115.26 ng	5.14 ng
Limit of Quantification (LOQ)	680.36 ng	16.69 ng

Table 3: Recovery study of piperine and glycyrrhetinic acid in pre-analyzed samples

Formulation	% of standard addition	Sample conc. Pip. : Gly A. (µg ml^{-1})	Piperine	Glycyrrhetinic acid
			* % Recovery ± SD	* % Recovery ± SD
Efiplus® Capsules	80	32.69 : 19.34	97.24 ± 1.08	94.68 ± 1.11
	100	32.69 : 19.34	98.11 ± 0.12	95.58 ± 2.26
	120	32.69 : 19.34	100.25 ± 1.17	98.62 ± 0.74

* Each value is mean ± standard deviation of three determinations

CONCLUSION

The developed HPTLC method has been shown to be selective, linear, precise and accurate. The results meet the guidelines of the International Conference on Harmonization (ICH) for validation of pharmaceutical assays of drug products. Amounts of glycyrrhetinic acid & piperine were found to be 31.98 ng/g and 77.156 ng/g respectively in marketed formulation. There was no interference in analysis of piperine and glycyrrhetinic acid from the other components present in the sample. The method was found to be simple, accurate and cost effective analytical method for routine analysis for two important markers in any polyherbal formulation.

REFERENCES

1. The Wealth of India, A Dictionary of Indian Raw materials and Industrial products, Publication and information Directorate, CSIR, New Delhi, 1976, vol XI, PP-89-96.
2. Bafna P.A. and Balaraman R., Antiulcer and antioxidant activity of pepticare (G. glabra, T. cordifolia and E. officinalis), a herbomineral formulation. *Phytomedicine.* V. 12(4) p. 264-270, 2005.
3. Bafna P.A. and Balaraman, Antioxidant activity of DHC-1, a herbal formulation in experimentally Induced cardiac and renal damage. *Phytotherapy Research.* V. 19(3) p. 216-221, 2005.
4. Dhanukar S.A., Zha A., Karandikar S.M., 'Antiallergic activity of Piper l ongum", *Indian journal of Pharmacology*, 1981, Vol. 13, P-122.
5. Kulshreshta V.K., Srivastava R.K., Singh N., Kohli R.P., 'A study of central stimulant effect of Piper longum", *Indian Journal of Pharmacology*, 1969, Vol. 1 (2) P-8.
6. Kulshreshta V.K., Srivastava R.K., Singh N., Rastogi R.K., Kohli R.P., "Analysis of central stimulant activity of Piper longum", *Journal of Research into Indian Medicine*, 1971, Vol. 6(1), P-17.
7. Roge N., Dhanukar S., Karandikar S.M., "Hepatoprotective effects of P.longum against carbon tetra chloride induced liver damage", *Indian Drugs*, 1984, Vol21, P-569.
8. Koul I.B., Kapi; A., "Evaluation of the Hepatoprotective potential of Piperine, an active principle of black and long peppers", *Planta Medica*, 1993, Vol 59(5), P-413.
9. Singh N., Kulshreshta V.K., Srivastava R.K., Kohli R.P., "Analeptic activity of some piper longum alkaloids", *Journal of Research into Indian medicine*, 1973, Vol. 8(1),P-1.
10. The Indian Materia Medica with Ayurvedic, Unani & Home Remedies, PP-965-968.

11. Tripathi D.M., Gupta N., Lakshmi V., Saxena K.C., Agarwal A.k., "Antigiardial and immunostimulatory effect of Piper longum on giardiasis due to Giardia lamblia" *Phytotherapy Research*, 1999, vol. 13 (7), P-561.
12. ICH-Guidelines Q 2A, Validation of Analytical procedures: Definition and Terminology (CPMP/ICH/381/95).
13. ICH-Guidelines Q 2B, Validation of Analytical procedures: Methodology (CPMP/ICH/281/95).

