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Development of HPTLC method for estimation of charantin in herbal formulations

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ABSTRACT - Charantin is one of the phytoconstituent present in *Momordica charantia* Linn. *M. charantia* is known for its hypoglycemic activity from ancient times. In the present study an attempt has been made to develop a HPTLC method for quantitative estimation of charantin in small, big, dried fruits used in formulations and different marketed antidiabetic polyherbal formulations (PHF). This HPTLC method was found to be reproducible, accurate and precise and detect charantin concentration at nanogram level. The developed HPTLC method would be an important tool in the quality control method of polyherbal formulations.

KEY WORDS - HPTLC, polyherbal formulations, charantin, antidiabetic

INTRODUCTION

Diabetes mellitus has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated(1). WHO has approved the use of traditional medicines as part of health programme. A herbal medicine is defined as a finished, labeled medicinal product that contains active ingredients as aerial or underground parts of plants or other plant material or combinations thereof. Approximately 3300 million people in the underdeveloped countries use medicinal plants on a regular basis while there has been great fascination for the herbal medicines and dietary food supplements in the developed countries(2). India has a rich heritage of usage of medicinal plants in the Ayurvedic, Siddha and Unani system of medicine with a mention of about 45,000 plants (3-4). A large number of plant preparations have been reported to possess antidiabetic activity over last several decades (5). A database of natural hypoglycemics collected by researchers in Mexico listed 800 plants. Researchers in India have documeted the use of over 150 plants in various families with hypoglycemic activity (6). A recent cross-cultural compendium cites over 1,200 medicinal plants used in diabetes (7). There are around 6000 herbal manufacturers in India. More than 4000 units are producing Ayurvedic medicines. Due to lack of Infrastructure, skilled manpower reliable methods and stringent regulatory laws most of these manufacturers produce their products on very tentative basis. Herbal drug formulations have received a raw deal from the modern medicine due to many reasons.

Momordica charantia Linn. Cucurbitaceae is a well known to possess antihyperglycemia, anticholesterol, immunosuppressive, antiulcerogenic, anti seperma -togenic and androgenic activities (8-9). One of the active principles reported to be responsible for various actions is charantin (10). All the three selected polyherbal formulations contain karela as one of the plants. The reference standard charantin had to be isolated, purified and the structure authenticated by various spectral analysis. There are no titrimetric, colorimetric, spectrophotometric or chromatographic methods available for quantitative estimation of charantin in different marketed antidiabetic PHFs. Therefore an attempt has been made to develop a HPTLC method because this method is fast, precise, sensitive and reproducible with good recoveries for standardization of polyherbal formulations.

MATERIAL AND METHODS

Equipment: A Cammag HPTLC system equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner III and Wincats an integration software 4.02 (Switzerland).

Chemicals: Analytical grade chloroform, benzene, methanol, formic acid ethyl acetate were obtained from S.D. Fine Chem Ltd (Mumbai, India). TLC aluminium plates pre-coated with silica gel 60 F 254 (10x 10 cms, 0.2 mm thick) used were obtained from E. Merck Ltd (Mumbai, India).

Sample preparation: Three polyherbal formulations were taken for the quantitative estimation of biomarkers like charantin, curcumin and swetiamarin. The selected PHFs were **Mersina** (J & J Dechane, Hyderabad), **Diabecone** (Himalaya Drug Co, Bangalore) and **Madhuripu** (LVG, Ahmedabad). 200 mg of these selected PHFs were taken for the quantitative estimation.

HPTLC method for estimation of charantin

Preparation of calibration curve of standard charantin

One milligram of working standard charantin was dissolved in 100 ml of chloroform to yield stock solution of 100µg/ml concentration. Calibration curve from 20-600 ng /spot was prepared and checked for reproducibility, linearity and validating the proposed method. The correlation coefficient, coefficient of variance and the linearity of results were calculated.

Sample preparation

200 mg of PHFs were taken and extracted in 10 ml of chloroform then the chloroform extract was filtered through Whatmann no. 42 filter paper. The final volume of the extract was made to 10ml with chloroform in volumetric flask. The charantin contents were analyzed after subjecting to HPTLC. The small and big karela were dried under shade and finely powdered. From that 50 mg of fine powder was taken and extracted by chloroform and filtered dried extract the volume make up to 2 ml with chloroform.

Method Specifications

Silica gel 60 F254 precoated plates (10 x 10 cm) were used with benzene: methanol (80:20) as solvent system. Sample was spotted on precoated TLC plates by using Linomat 5 spotter. Ascending mode was used for development of thin layer chromatography. TLC plates were developing up to 8 cms. The plates were sprayed with 10% sulphuric acid in alcohol and the reagent was prepared freshly, heated at 130^0 C for 2-3 min and brought to room temperature. Violet spot with Rf 0.32 was visible and scanned under 536 nm. The contents of charantin in the selected PHFs were determined by comparing area of the chromatogram of PHFs with calibration curve of the working standard of charantin.

RESULTS AND DISCUSSION

Standard charantin showed single peak in HPTLC chromatogram. The calibration curve of charantin was obtained by spotting standard charantin on HPTLC plate. After development the plate was scanned at 536 nm (Fig.1). The calibration curve was prepared by plotting the concentration of charantin versus average area of the peak (Fig.2). PHFs were analysed by the proposed method. The amount of charantin was computed from calibration curve and calibration curve was shown in Fig. 2.

Figure 1: HPTLC chromatogram of standard charantin

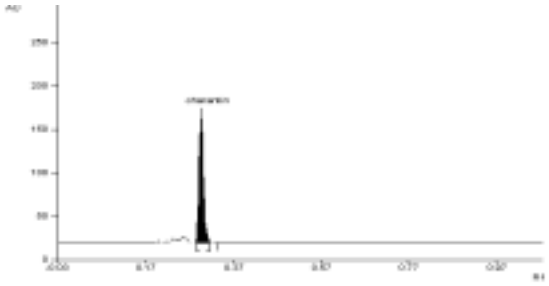
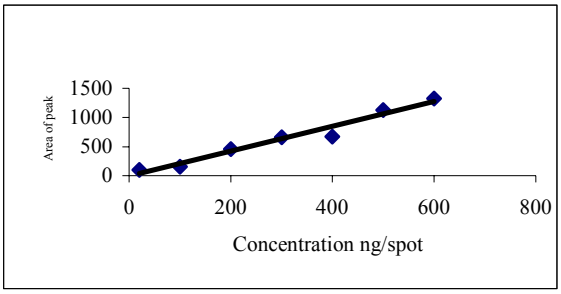


Figure 2: Calibration curve of peak area versus standard charantin concentration ranging from 20ng- 600ng/spot



Data from Table 3 revealed that Madhuripu contained highest quantity of the charantin. We found little less amount of charantin in Diabecone and Mersina. It may be due to varied factors like time of collections, age of the pant, processing conditions, incorrect identification of the plant, improper selection of the herb variety, addition of exhausted material and genetic variety of the plat material.

Validation of HPTLC method

1. Linearity: A representative calibration curve of charantin was obtained by plotting peak area of charantin against the conc4ntration of charantin over the range of 20- 600 ng/spot. The correlation coefficient was found to be 0.9635.

2. Accuracy (% Recovery):

The % recovery of charantin given in Table 3 was found to be 98.89 which is satisfactory.

3. **Specificity:** It was observed that the other herbs present in the formulations their constituents an excipients did not interfere with the peak of charantin. Therefore the method was specific. The spectrum of standard charantin spot and charantin in other sample was found to be similar or overlap.

Table 1: Linear range, co-relation coefficient and standard deviation for charantin

Parameters	Values
Linear range	20-600 ng
Standard Deviation (According to area)	3.752762
Correlation coefficient (According to area)	0.9635

Table 2: Percentage of charantin in different type of M.charantia fruits and its formulations by measuring area in HPTLC method

Sample	Amount of charantin per ng/spot	%w/w charantin
Small Karela	301	0.602
Big karela	235.88	0.4716
Fresh fruits	193.15	0.3863
Madhuripu	375.21	0.09356
Diabecone	257.68	0.06443
Mersina	252.18	0.06305

Table 3: Results of recovery study of the method

Amount of charantin added in ng	Amount of charantin found (ng)	% Recovery	Average recovery
50	49.63±0.48	99.26	
100	98.87±1.06	98.87	98.89
150	147.8±0.96	98.53	

4. Limit of Detection: The minimum detectable limit was found to be 20 ng / spot.

CONCLUSIONS

The proposed HPTLC method was found to be rapid, simple and accurate for quantitative estimation of charantin in different marketed polyherbal formulations and small fruits, big fruits of *M. charantia*. The recovery values of charantin were found to be about 98.89%, which shows the reliability and suitability of the method. The lowest detectable limit of charantin in different formulations was found upto 20 ng/spot.

REFERENCES

1. R.D. Leslie, R.B. Elliott, Early environmental events as a cause of IDDM. *Diabetes*. **43:** 843 (1994).
2. P.J. Campbell, M.G. Carlson, Impact of obesity on insulin action in NIDDM. *Diabetes*. **42:** 405 (1993).
3. U. Smith, Insulin resistance in obesity, type II diabetes and stress. *Acta Endocrin supp*. **262:** 67 (1984).
4. M. Krotkiewski, P. Bjrntorp, U. Smith, Impact of obesity on metabolism in men and women. *J Clin Invest*. **72:** 1150 (1983).
5. J.A. Marshall, R.F. Hamman, J. Baxter, High-fat, low-carbohydrate diet and the etiology of non-insulin-dependent diabetes mellitus. *Am J Epidemiol*. **134:** 590 (1991).
6. J.A. Marshall, Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. *Diabetes Care*. **17:** 50-6 (1994).
7. R. Riales, M. Albrink, Effect of chromium chloride supplementation on the glucose tolerance and serum lipids, including HDL, of adult men. *Am J Clin Nutr*. **34:** 2670-8 (1981).
8. P. Scartezzini, E. Speroni, Review on some plants of Indian traditional medicine with antioxidant activity. *J Ethnopharmacol*. **71(1-2):** 23- 43 (2000).
9. A. S. Bourinbaiar, S. Lee-Huang, Potentiation of anti-HIV activity of anti- inflammatory drugs, dexamethasone and indomethacin, by MAP30, the antiviral agent from bitter melon. *Biochem. Biophys. Res. Commun*. **208(2):** 779-85 (1995).
10. G.V. Senanayake, M. Maruyama, K.Shibuya, M. Sakono, N. Fukuda, T.Morishita, C.Yukizaki, M. Kawano, H. Ohta, The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats, *Journal of Ethnopharmacology*. 91(2): 257-262 (2004).