A validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing *Trigonella foenum-graecum* (L.) seeds

**Abstract**

**Background:** *Trigonella foenum-graecum* (L.) (Fabaceae, Fenugreek) is an important ingredient of Ayurvedic and other marketed herbal formulations. Fenugreek seeds are employed in many traditional systems as an antibacterial and antidiabetic agent, gastric stimulant and galactogogue. Trigonelline, a major phytoconstituent found in fenugreek seeds, shows estrogenic, anti-diabetic and anti-invasive activity. Therefore, it is a suitable bioactive marker to establish the quality of crude drug and its formulations. **Objective:** To develop an efficient and effective RP-HPLC method for estimation of trigonelline from *Trigonella foenum-graecum* seeds and its marketed herbal formulations. **Materials and Methods:** Separation and detection of trigonelline was carried out on a Cosmosil CN-MS column eluted with methanol:distilled water [95:5, v/v; pH 3.5 using hydrochloric acid]. Detection was carried out at 267 nm using a Photo Diode Array detector. Fenugreek seeds and two marketed herbal formulations were subjected for HPLC analysis of Trigonelline. **Results:** The RP-HPLC method was validated as per ICH guidelines and the content of trigonelline in marketed polyherbal formulations such as Dibet powder and Amyron syrup was determined. The LOD and LOQ were found to be 5.00 ng/mL and 50.00 ng/mL, respectively. Detector response was linear from 100.00 to 8000.00 ng/mL. The method was found to be simple, sensitive, accurate, reproducible and rugged. **Conclusion:** This work can be recommended for quality assurance and marker-based standardization of formulations containing fenugreek seeds. **Key words:** Amyron syrup, dibet powder, RP-HPLC, *Trigonella foenum-graecum* (L.), trigonelline

**INTRODUCTION**

*Trigonella foenum-graecum* (L.) (Fabaceae), commonly known as Fenugreek, is an aromatic and annual herb cultivated throughout the country. Fenugreek seeds are sharp bitter in taste and possess antipyretic, anthelmentic, antileprotic, antibronchitic, carminative and aphrodisiac properties. Several confections made with the seeds are used as a remedy for dyspepsia, loss of appetite, diarrhea of puerperal women and in rheumatism.[1,2]

The main chemical constituents of *Trigonella foenum-graecum* are fibers, flavonoids, polysaccharides, saponins, flavonoids, fixed oils, and some identified alkaloids namely, trigonelline and choline.[2] Trigonelline [Figure 1] is an important bioactive marker with estrogenic, anti-diabetic, and anti-invasive properties.[3-5] There are several chromatographic methods reported for quantification of trigonelline from pumpkin fruit, coffee seed etc. Most of these methods have issues such as the use of no organic phase, varied flow rate, costly columns (polymer based), and gradient elution which make them cumbersome for implementation in QC laboratories.[6-12]

This study aims at optimizing and validating a simple and reliable HPLC
Materials and methods

The extraction method on a reversed-phase Cosmosil CN-MS (250 mm × 4.6 mm) column with photodiode array (PDA) detection for monitoring the quality of *Trigonella foenum-graecum* seed.[13-15] The developed method was also successfully applied to commercially available polyherbal formulations (Dibet powder and Amyron syrup) to estimate relative trigonelline content.

**HPLC analytical conditions**

HPLC analysis was performed on a JASCO's HPLC system equipped with a PU-980 pump unit, a reversed-phase Cosmosil (Nacalai Tesque, INC. Japan) CN-MS (250 mm × 4.6 mm) column, an autosampler (AS-1555-10), and a Photo diode array detector (MD-910). Samples were eluted using the mobile phase of methanol:distilled water (95:5, v/v), adjusted to pH 3.5 with hydrochloric acid and delivered at a flow rate of 1.0 ml/min. Detection was carried out at 267 nm at room temperature (27 ± 1 °C). The injection volume was 20 µL for all runs. Data acquisition and analysis were carried out using Borwin Integrator Software, version 1.21, chromatography analysis software.

**Standard solution**

Trigonelline (10 mg) was dissolved in 10 mL of methanol to prepare a stock solution of 1000 µg/mL. Working standard solution was prepared by serial dilution of the standard stock solution.

**Method validation**

The developed RP-HPLC method was validated as per ICH guidelines in terms of its sensitivity (LOD and LOQ), linearity, assay, recovery, precision, stability, and ruggedness/robustness.

**RESULTS AND DISCUSSION**

Currently chemical markers or pharmacologically active components in polyherbal formulations are employed for evaluating the quality, consistency, and authenticity of polyherbal formulations.[16,17] Based on the experiments carried out during the course of validation, the intended method has been validated for the estimation of trigonelline from seeds of *Trigonella foenum-graecum*. The precision and accuracy were within the acceptance of limits. Consistent recoveries were observed for LQC, MQC, and HQC (lower, middle and higher quality control samples respectively). Stability of stock and working standard were checked for short term (6 h) as well as long term (2-3 months) storage at 4 ± 1 °C.
Table 1: Method validation parameters

| Parameters                   | Results          |
|------------------------------|------------------|
| LOD (ng/mL)                  | 5.0              |
| LOQ (ng/mL)                  | 50               |
| Linear range (ng/mL)         | 100–8000         |
| Mean correlation coefficient ($r^2$) | 0.9967          |
| Mean slope                   | 22.412           |
| System suitability (% CV, $n = 5$) | Retention time 0.13 |
|                              | Area             | 1.05             |
| Precision (% CV, $n = 3$)    | Within-batch    | 1.14–1.73        |
|                              | Between-batch   | 1.69–1.96        |
| Recovery (%, $n = 7$)        | LQC              | 101.80           |
|                              | MQC              | 99.24            |
|                              | HQC              | 98.67            |
| Stability                    | Long-term stability | Stable at (4 ± 1°C) |
|                              | Standard stock solution stability | Stable at (4 ± 1°C) |
|                              | Short-term stability | Bench top stability (For 6.00 h) Stable at (25 ± 2°C) |
|                              |                  | Autosampler stability (For 12.00 h) Stable at (4 ± 1°C) |

Figure 2: Representative HPLC chromatograms of (a) standard trigonelline 1000.0 ng/mL, (b) *Trigonella foenum-graecum* (L.) seeds, (c) Dibet powder and (d) Amyron syrup.

as for long term (10 days). The stock standard was found to be stable at 4 °C for 10 days [Table 1]. The bench top stability and autosampler stability for the working standard showed stability for 6.00 h.
CONCLUSION

As herbal preparations have chemical complexities, it is very difficult to identify and determine all of their chemical components. Single marker-based quantitative methods would be a complementary approach for the quality control and stability assessment of the herbal preparations. Our results provide a fully validated RP-HPLC method for quality control of plant extracts and phytopharmaceuticals containing seeds of *Trigonella foenum-graecum* using trigonelline as a chemical marker. Validation of the method as per ICH guidelines showed that the method is in compliance with the current guideline. The method was found to be robust. Moreover, the solvent consumption along with the short analytical run time of 8.0 min leads to a cost-effective and eco-friendly chromatographic procedure. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure and represents a good procedure for quantitation of trigonelline.

ACKNOWLEDGMENTS

We acknowledge the financial assistance from NMPB, Government of India (Project No. GO/MH-04/2009) for carrying out this work. We are also thankful to Mr. Bhavesh Tiwari and Mr. Harshvardhan Joshi for their technical assistance.

REFERENCES

1. Mehrafarin A, Rezazadeh SH, Naghdi BH, Noormohammadi GH, Qaderi A. A review on biology, cultivation and biotechnology of fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant and multipurpose. J Med Plants Res 2011;5:10-6.
2. Toppo FA, Akhand R, Pathak AK. Pharmacological actions and potential uses of *Trigonella foenum-graecum*: A review. Asian J Pharm Clin Res 2009;2:29-38.
3. Yoshinari O, Sato H, Igarashi K. Anti-diabetic effects of pumpkin and its components, trigonelline and nicotinic acid, on Goto-Kakizaki rats. Biosci Biotechnol Biochem 2009;73:1033-41.
4. Hiramaka N, Okuuchi R, Miura Y, Yagasaki K. Anti-invasive activity of niacin and trigonelline against cancer cells. Biotech Biochem 2005;69:653-8.
5. Allred KE, Yackley KM, Vanamala J, Allred CD. Trigonelline is a novel phytoestrogen in coffee beans. J Nutr 2009;139:1833-8.
6. Casal S, Oliveira MB, Ferreira MA. Development of an HPLC/Diode-Array detector method for simultaneous determination of trigonelline, nicotinic acid, and caffeine in coffee. J Liq Chromatogr Relat Technol 1998;21:3187-95.
7. Zhang XH, Zhao HQ, Qu Y, Wang XY, Lu XY, Hattori M. Determination of trigonelline by HPLC and study on its pharmacokinetics. Yao Xue Xue Bao 2003;38:279-82.
8. Zhao HQ, Qu Y, Wang XY, Zhang HJ, Li FM, Masao H. Determination of trigonelline in *Trigonella foenum-graecum* by HPLC. Zhongguo Zhongyao Zazhi 2002;27:194-6.
9. Qin XY, Chong W, Shi CC. HPLC determination of trigonelline in pumpkin pulp. Food Sci 2010;31:209-11.
10. Guangxue L, Mingying S, Hui L, Shaqing C. Extraction and determination of trigonelline in *Trigonella foenum-graecum* seeds. Drug Standards China 2005;4:11-4.
11. Rongjie Z, Li W, Longxing W, Hongxin X, Shaqing C. Determination of trigonelline in *Trigonella foenum-graecum* by hydrophilic interaction chromatography. Chin J Chromatogr 2010;28:379-82.
12. Chun ZY, Xin Y, Hua Z, Jing W, Chang XD. HPLC determination of trigonelline in pumpkin powder. Food Sci 2008;1:280-2.
13. EMEA. Note for Guidance on Quality of Herbal Medicinal Products. The European Agency for the Evaluation of Medicinal Products: London; 2001.
14. USFDA. Guidance for Industry Botanical Drug Product. U.S. Food and Drug Administration: Rockville; 2004.
15. Guidelines for the Assessment of Herbal Medicine World Health Organization. Geneva: WHO; 1991.
16. Cabrito JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000;33:179-89.
17. Liang YZ, Xie P, Chan K. Quality control of herbal medicines. J Chromatogr B Analyst Technol Biomed Sci 2004;812:53-70.

How to cite this article: Shailajan S, Menon S, Singh A, Mhatre M, Sayed N. A validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing *Trigonella foenum-graecum* (L.) seeds. Pharm Methods 2011;2:157-60.

Source of Support: NMPB, Government of India (Project No. GO/MH-04/2009).

Conflict of Interest: None declared.

### Table 3: Assay results and method application

| Sample              | Concentration of trigonelline in mg/ml or mg/g (mean ± SD) |
|---------------------|------------------------------------------------------------|
| *Trigonella foenum-graecum* | 98.36 ± 0.0154                                             |
| Amyron syrup        | 0.025 ± 0.0011                                              |
| Dibet powder        | 0.015 ± 0.0026                                              |

(25 ± 20°C) and for 12.00 h (4 ± 10°C) respectively [Table 1]. The validated method was also applied for quantification of trigonelline from two marketed herbal formulations Dibet powder and Amyron syrup containing seeds of *Trigonella foenum-graecum* [Figure 2]. The method has been found to be rugged for different columns, different analysts, different days, change in the instrument (PEV Jasco), change in the flow rate, change in injection volume, and variation in the mobile phase composition under specified conditions [Table 2]. The representative chromatograms and content of trigonelline in seeds of *Trigonella foenum-graecum*, Amyron syrup and Dibet powder are given in Figure 2 and Table 3 respectively.