

Original Research Article

Simultaneous Determination of Co-formulated Matrine and Secnidazole in Suppositories by Reverse Phase High Performance Liquid Chromatography

Haixia Lv, Suying Ma*, Xiao Wang and Xiaojun Shang

School of Pharmacy, Xinxiang Medical University, Xinxiang Henan, 453003, PR China

*For correspondence: **Email:** suyingma@163.com; **Tel:** + 86-373-3029879

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Abstract

Purpose: To develop and validate a new, simple, sensitive and accurate high performance liquid chromatographic (HPLC) method for the simultaneous determination of matrine and secnidazole in suppositories.

Methods: The method involved using a SinoChoom ODS-BP C₁₈ column (5 µm, 4.6 mm x 200 mm) and mobile phase consisting of acetonitrile–triethylamine (0.05 %) in 0.025 mol/L KH₂PO₄ (20:80, v/v). The flow rate was 1 mL/min and detection was monitored at 210 and 311 nm for matrine and secnidazole, respectively. Total run time was 10 min and the column was maintained at 35 °C.

Results: The excipients in the suppository did not interfere with the drug peaks. Matrine was eluted at a retention time (RT) of 4.30 min while linearity for the quantification of drug was obtained in the concentration range of 10.0 - 100.0 µg/mL ($r^2 = 0.9991$). Secnidazole was eluted at a retention time (tr) of 6.69 min and linearity for the quantification of the drug was obtained in the concentration range of 10.0 - 150.0 µg/mL ($r^2 = 0.9993$). Intra- and inter-day variations were < 1.0 % for both matrine and secnidazole.

Conclusion: The developed HPLC method was validated according to International Conference on Harmonisation (ICH) guidelines and proved to be suitable for the simultaneous determination of matrine and secnidazole in suppositories.

Keywords: Matrine, Secnidazole, Suppository, HPLC, Assay

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INTRODUCTION

Bacterial vaginosis is a common cause of malodorous vaginal discharge in women of reproductive age, occurring in up to 30 % of women [1]. Besides being unpleasant for patients when symptoms of discharge and odour occur, bacterial vaginosis is associated with increased risk of several pathological gynaecological conditions as well as major adverse outcomes during pregnancy. It is estimated that bacterial vaginosis is associated with a two-fold increased

risk of pre-term birth and a six-fold increased risk of miscarriage [2]. Bacterial vaginosis is also associated with increased susceptibility to sexually transmitted infections, including herpes simplex viruses, human papillomavirus, and human immunodeficiency virus[3,4].

Secnidazole, chemically 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole (Fig.1), is a new second generation 5-nitroimidazole product [5]. It is a nitroimidazole antibiotic with a broad spectrum of

activity against anaerobic microorganisms, higher efficacy, fewer adverse effect and has a longer half-life than metronidazole. It was widely used for the treatment and prevention of certain bacterial and protozoal diseases clinically. Used as a single-dose oral regimen, it appears effective in the treatment of amebiasis, giardiasis, trichomoniasis, and bacterial vaginosis. [6,7]

Matrine, chemically 7aS,13aR,13bR,13cS)-dodecahydro-1H,5H,10H- dipyrido [2,1- f:3',2',1'- ij] [1,6] naphthyridin-10-one (Fig. 1), is an important ingredient extracted from *Sophora flavescens* Ait, *Sophora subprostrata*, and *Sophora alopecuroides* L., which are leguminous plants widely distributed in the northwestern region of China and are important Chinese herbal remedies. It has been proved that matrine is a nitrogen-containing heterocyclic, which belong to the quinolizidine alkaloids. It is very soluble in water, chloroform and benzene [8-12]. It has a wide range of pharmacological applications including anti-inflammatory, antiendotoxic and anticancer with no obvious toxicity or side effect [13]. The suppository combining matrine and secnidazole can play synergistic roles for effective treatment of gynecological diseases.

High-performance liquid chromatography (HPLC) has been widely used to quantitate matrine and secnidazole individually in preparations and biological samples. To the best of our knowledge, few HPLC methods have been developed in the literature for the determination of matrine and secnidazole in preparations simultaneously [14-19]. The literature was mainly focused on determination of the content of matrine and secnidazole one by one. The aim of this study is to develop and validate a simple, rapid, sensitive and reproducible HPLC method to determine matrine and secnidazole simultaneously when combined in a suppository with the advantages of shorter retention time and run-time.

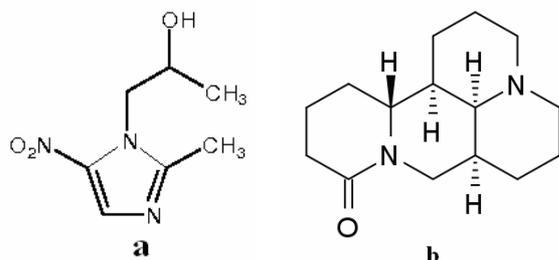


Figure 1: Typical chemical structure of (a) secnidazole and (b) matrine

EXPERIMENTAL

Materials

Pure matrine and secnidazole used as working standards were received as gifts from Zhongzhou Pharmaceuticals Co., Ltd, (Zhengzhou, PR China). Acetonitrile of HPLC grade and other chemicals of AR grade were purchased from Mingshen Chemicals Co, Ltd, (Zhengzhou, PR China). Deionized water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals were used as received without further purification.

Preparation of matrine/secnidazole suppositories

Matrine/secnidazole suppositories were prepared by fusion method. Mixtures of gelatin, glycerin and water (10:15:15, w/v) were used as suppository bases; the mixture was placed in a beaker and melted by heating to 70 °C in a hot water bath. Secnidazole (16 g) and matrine (4 g) were mixed with the molten base, poured into a 10 holes steel suppository mold and allowed to solidify at room temperature. After solidification, the formed suppositories were removed from the mold, wrapped with aluminum foil, and stored in a desiccator with silicone in the refrigerator at 4 °C pending further use. Each of the suppositories was formulated to contain 200 mg secnidazole and 50 mg matrine.

Determination of matrine and secnidazole

Chromatographic analysis was performed using a Shimadzu system that comprised of LC-20AT pump, SPD 20A UV-visible absorbance detector connected to Shimadzu Spin Chrome software. ODS-BP C₁₈ column (5 μm, 4.6 mm×200 mm) was used and the sample injection was performed via a Rheodyne syringe.

The mobile phase was a mixture of acetonitrile-triethylamine (0.05 %) in 0.025 mol/L KH₂PO₄ (20:80, v/v). The flow rate was 1.0 mL/min. The mobile phase was degassed by an ultrasonic bath and filtered through a 0.45μm membrane filter under vacuum. The eluents were detected and quantified at 210 nm from 0 min to 5.5 min and at 311nm from 5.5 min to 10.0 min. The column was maintained at 35 °C and injection volume was 20 μL.

Preparation of standard stock solutions and working solutions

The standard solutions of the pure drugs were prepared by dissolving accurately 100 mg matrine and 150 mg secnidazole in separate 10 mL volumetric flasks using the mobile phase, respectively. The volumes were made up to the mark with mobile phase. Intermediate and working solutions were prepared by diluting stock solutions with the mobile phase. Calibration standard solutions were prepared in the concentration range of 10 to 100 µg/mL for matrine and 10 to 150 µg/mL for secnidazole, and injected into the system in triplicate. The chromatogram peak area of each drug concentration was calculated. The regression of the drug concentration versus the peak area was obtained.

Quantification of matrine and secnidazole in suppository

Ten suppositories were accurately weighed and crushed to fine particles in a mortar and an amount equivalent to one suppository was transferred into a 100 mL volumetric flask. The mixture was dissolved and then made up to volume with mobile phase. It was kept in an ultrasonic bath for 5 min and the solution filtered through a 0.45 µm filter paper. Suitable aliquots of the filtered solution were transferred to a volumetric flask and made up to volume with mobile phase to yield concentrations of matrine (20.0 µg/mL) and secnidazole (80.0 µg/mL). A 20 µL aliquot of the sample solution was injected into the chromatographic system three times under optimized chromatographic conditions. The peak area was measured at 210 nm from 0 to 5.5 min and at 311 nm from 5.5 to 10.0 min for matrine and secnidazole, respectively. Drug concentrations of the samples were determined by interpolation from calibration plots of each drug previously obtained.

Method validation

The method was validated with parameters of specificity, linearity, accuracy, precision, reproducibility and robustness.

Specificity

Combined working solution (matrine 20.0 µg/mL, secnidazole 80.0 µg/mL), blank excipients sample without matrine and secnidazole, equal concentrations sample of combined suppository (matrine 20.0 µg/mL, secnidazole 80.0 µg/mL) made with above procedure were scanned from

200nm to 400nm, and then the chromatograms were recorded.

Linearity

Matrine and secnidazole working solutions at eight different concentrations (The concentration was 10, 25, 35, 45, 55, 75, 85, 100 µg/mL for matrine and 10, 20, 40, 50, 80, 100, 120, 150 µg/mL for secnidazole, respectively) were prepared for linearity studies and injected into chromatographic system (n = 3). The responses were measured as peak area.

Accuracy

Adequate amounts of matrine and secnidazole were added to gelatin and glycerin excipients to make three concentration levels (80, 100 and 120 %). At each level, three determinations were performed and the results were recorded. Accuracy was expressed as percent analyte recovered by the proposed method.

Precision

The precision of the method was checked by repeatability of injection, repeatability (intra-day), intermediate precision (inter-day) and reproducibility. Injection repeatability was studied by calculating percent relative standard deviation (% RSD) for ten determinations each of peak area of matrine (20.0 µg/mL) and secnidazole (80.0 µg/mL) performed on the same day. The same solutions were injected in triplicate for both intra-day and inter-day variation

Robustness

The robustness of the developed method was determined by carrying out the analysis, during which mobile phase composition (concentration of acetonitrile was varied by ± 5 %), flow rate and column temperature were altered and the peak areas and retention times were recorded.

RESULTS

Specificity

From the UV-visible spectra, matrine had maximum absorption at 210 nm and secnidazole had maximum absorption at 311 nm. Thus, 210 nm and 311 nm were selected as detection wavelengths. Under optimum conditions, typical chromatograms of matrine and secnidazole solutions, blank excipients sample without matrine and secnidazole, suppository sample are shown in Fig 2. The retention times of matrine and secnidazole at a flow rate of 1.0 mL/min

were 4.30 and 6.69 min, respectively. Analyte peaks were well resolved and free from tailing (< 1.5 for both analytes). The excipients in the suppository did not interfere with the detection of matrine and secnidazole.

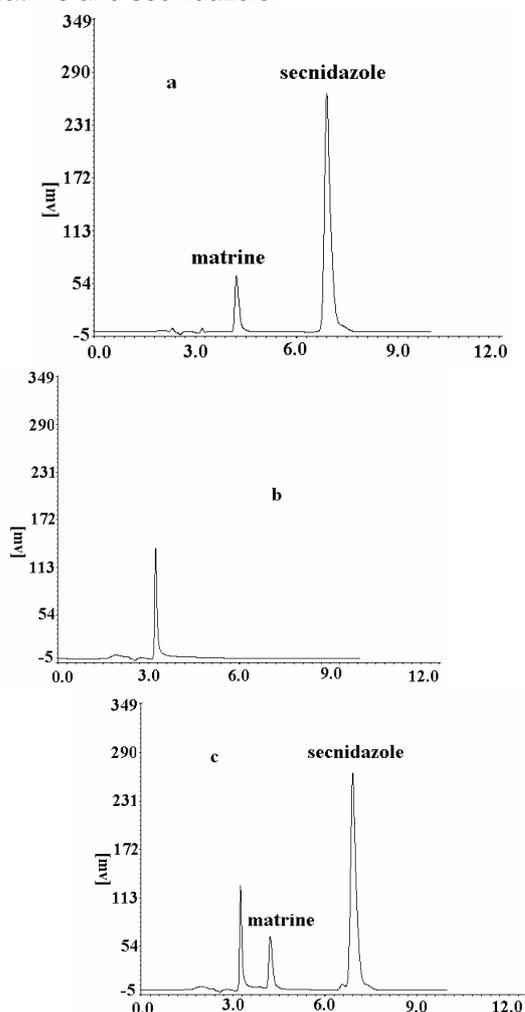


Figure 2: Chromatograms of (a) matrine ($\hat{RT} = 4.30$) and secnidazole ($\hat{RT} = 6.69$); (b) blank gelatin and glycerin excipients sample, and (c) suppository sample

Linearity

Under optimal conditions, the calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10.0 - 100.0 $\mu\text{g/mL}$ for matrine and 10.0 - 150.0 $\mu\text{g/mL}$ for secnidazole. The regression equations, $y = 28.266x + 25.017$ for matrine and $y = 43.774x - 12.475$ for secnidazole were established based on the standard samples injected, with correlation coefficients of 0.9991 and 0.9993 respectively; where y is peak area based on three parallel measurements and x is the concentration ($\mu\text{g/ml}$) of matrine or secnidazole standard solution. The correlation coefficients indicate a good linear

relationship between peak area and concentration over a wide range.

Accuracy

Mean recovery for matrine and secnidazole was 99.88 and 100.13 %, respectively.

Precision

The measurements of intra- and inter-day variability were utilized to determine the precision of the developed method. The relative standard deviation (RSD) of intra-day variation for matrine was 0.75 % and for inter-day variation 0.81% while the corresponding values for secnidazole were 0.69 and 0.72 %. The low values of RSD indicate that the method was precise. Injection repeatability values of matrine and secnidazole were 0.87 and 0.64 %, respectively. Reproducibility was checked by having the samples analyzed by another analyst using same instrument and same laboratory. There was no significant difference between the RSD values, which indicate that the proposed method was reproducible.

Robustness

The robustness test results for the developed method are as shown in Table 1. It is clear that there was no significant change in peak area and retention time of matrine and secnidazole when the ratio of acetonitrile, flow rate and column temperature were varied ($p < 0.05$). The results indicate that the developed method was sufficiently robust for normally expected variations in chromatographic conditions.

Content of matrine and secnidazole in suppositories

Matrine and secnidazole, when co-formulated in suppositories, were simultaneously determined with the proposed method. The results of the assay yielded 100.07 ± 0.45 % for matrine and 99.92 ± 0.38 % for secnidazole, thus showing that the method was selective and accurate for the simultaneous determination of matrine and secnidazole without interference from the excipients in the suppository dosage form.

DISCUSSION

This study was essentially focused on the simultaneous determination of co-formulated matrine and secnidazole in suppositories. Following initial trials to establish optimum conditions, a mixture of acetonitrile-triethylamine (0.05%) in 0.025 mol/L KH_2PO_4 (20:80, v/v) was

Table 1: Method robustness test results for matrine and secnidazole

| System suitability parameter | Analyte | Variation in acetonitrile content of mobile phase | | | Variation in flow rate (mL/min) | | | Variation in column temperature (°C) | | |
|------------------------------|-------------|---|-----|-----|---------------------------------|-----|-----|--------------------------------------|-----|-----|
| RSD (%) | Matrine | 19 | 20 | 21 | 0.9 | 1.0 | 1.2 | 34 | 35 | 36 |
| | | 0.2 | 0.1 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.3 |
| | Secnidazole | 19 | 20 | 21 | 0.9 | 1.0 | 1.2 | 34 | 35 | 36 |
| | | 0.2 | 0.1 | 0.3 | 0.2 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 |

selected as the optimum mobile phase for baseline separation and short retention time.

Matrine and secnidazole were soluble in organic solvents including methanol and acetonitrile. The use of mobile phase as extraction reagent provided minimal impurities and better separation.

The successfully validated method using HPLC grade solvents had short retention times and high peak symmetry. Therefore, it can be performed in any laboratory with adequate HPLC instrumentation for a relatively rapid and low-cost assay. The mobile phase system gave sharp peaks for matrine and secnidazole without interfering peaks. The retention time (RT) of matrine and secnidazole was 4.30 and 6.69 min, respectively. The method was linear over wide concentration ranges for matrine and secnidazole, indicating good correlation between concentration and peak area.

The method was validated in terms of linearity, accuracy, precision and robustness according to ICH guidelines. Adequate resolution between matrine and secnidazole peaks showed the efficiency of the method based on its capacity to identify and determine each analyte at the same time with no interference. The method was robust because minor changes in the chromatographic parameters did not bring about any significant changes in peak area and retention time. The accuracy data show that the method is accurate within the desired ranges.

CONCLUSION

The developed method is simple, rapid, cost-effective, highly sensitive and provides good reproducibility and accuracy for the simultaneous determination of matrine and secnidazole when co-formulated in suppositories.

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