Simultaneous determination of triacetin, acetic ether, butyl acetate and amorolfine hydrochloride in amorolfine liniment by HPLC

Yuan Gao[1], Li LI[2], Jianjun Zhang[3*], Wenjuan Shu[1] and Liqiong Gao[1]
[1]School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, PR China
[2]No.2 Affiliated Hospital of Nanjing Medical University, Nanjing, PR China
[3]School of Pharmacy, China Pharmaceutical University, Nanjing, PR China

Abstract: A simple, rapid, specific and precise reversed-phase high-performance liquid chromatographic method was developed for simultaneous estimation of triacetin, acetic ether, butyl acetate and amorolfine in marketed pharmaceutical liniment. Chromatographic separation was performed on a Shimadzu VP-ODS C18 column using the mixture of citric acid-hydrochloric acid-sodium hydrate buffer (pH 3.0), acetonitrile and methanol (32:30:38) as the mobile phase at a flow rate of 1.0 mL/min with UV-detection at 215 nm. The method separated the four components simultaneously in less than 10 min. The validation of the method was performed with respect to specificity, linearity, accuracy, and precision. The calibration curves were linear in the range of 35.1-81.9 μg/mL for triacetin, 431.1-1005.9 μg/mL for acetic ether, 167.0-389.7 μg/mL for butyl acetate and 151.0-352.3 μg/mL for amorolfine. The mean 100% spiked recovery for triacetin, acetic ether, butyl acetate and amorolfine is 99.43 ± 0.42, 101.5±1.09, 101.4±1.02 and 100.8±0.69, respectively. The intra-day and inter-day relative standard deviation values were <2.0%. The limits of detection of these compounds ranged from 0.08 to 5.88 ng. The utility of the procedure was verified by its application to the commercial liniment.

Keywords: Amorolfine, liniment, HPLC, triacetin, acetic ether, butyl acetate.

INTRODUCTION

Amorolfine, cis-2, 6-Dimethyl-4-[2-methyl-3-(p-tert-pentylphenyl) propyl] morpholine, is an allylamine antifungal drug, which depletes ergosterol and causes ignosterol to accumulate in the fungal cytoplasmic cell membranes (Clayton, 1994; Polak, 1992). It has a broad spectrum of activity, including dermatophytes, various filamentous and dematiaceous fungi, yeasts and dimorphic fungi. Its activity is fungicidal for most species (Haria and Bryson, 1995).

Marketed liniment containing 5% amorolfine as the active ingredient is commercially available to clinically treat the infections of the nails with fungi (dermatophytes), yeasts and moulds (Reinel and Clarke, 1992; Pittrof *et al*., 1992; Zang and Bergstraesser, 2006). As stated in the dispensatory of the marketed products (Curanail® and Loceryl®), it also contains triacetin, acetic ether, butyl acetate, alcohol and acrylic resin. In the liniment, triacetin works as a plasticizer to improve the smoothness, integrity and continuity of the films; acetic ether and butyl acetate are volatile organic solvents to help acrylic resin build a water insoluble film on the nail plate which remains at the application site for a week and promotes drug to infiltrate into the nail plate (Pittrof *et al*., 1992). Triacetin, acetic ether and butyl acetate are crucial for the formulation, production and clinical effectiveness of the liniment product.

**Corresponding author:* e-mail: highmorepharm@gmail.com

An HPLC method was reported only for the assay of amorolfine in its liniment (Wang and Yu, 2000). Triacetin, acetic ether or butyl acetate were not mentioned in the report. Comparative study showed that the reported HPLC procedure can not get good detection and separation among the four components. HPLC analysis of acetic ether (Zhang, 2008) and GC (Wang, 2007) analysis of butyl acetate can be found in literatures. However, there is no method reported dealing with the simultaneous determination of all four components (triacetin, acetic ether, butyl acetate and amorolfine) in pharmaceutical products so far.

The aim of the present study was to develop a simple, rapid, specific and precise RP-HPLC method for the simultaneous determination of four components and the quality control of liniment. The validation of the proposed method was also carried out and its applicability was evaluated in commercial liniment analysis.

MATERIALS AND METHODS

Materials and reagents

Amorolfine hydrochloride liniment (Loceryl®, lot: 8212156, Laboratories Galderma, France) containing 50 mg amorolfine per milliliter liniment and unknown amount of triacetin, acetic ether and butyl acetate was analyzed. Amorolfine hydrochloride reference standard (99.9% of purity) was obtained from International Laboratory, USA. Reference standards of triacetin (99.5% of purity), acetic ether (99.9% of purity) and butyl acetate (99.7% of purity) were obtained from Dior, Germany. For

recovery studies, triacetin, acetic ether, butyl acetate and amorolfine were purchased from Shuyi Chemical Reagent, Shanghai, China. Ethanol was provided by Anhui Ante Biological Chemistry, China and Acrylic resin was provided by Degussa, Germany.

Acetonitrile and methanol (HPLC grade) were purchased from Merck, Germany. All other chemicals were of analytical grade and were used without further purification. The citric acid-hydrochloric acid-sodium hydrate buffer was prepared as follows: 0.63 g of citric acid, 0.6 mL of hydrochloric acid and 0.24 g of sodium hydrate were transferred into 100 mL volumetric flask, shaken until dissolved and diluted to volume with water. This solution was mixed with water and triethylamine (25:175:0.3, v/v/v), and pH was then adjusted to 3.0 by hydrochloric acid.

Instrumentation and chromatographic conditions

The HPLC analysis was performed on HPLC-LC-2010 AHT (Shimadzu, Japan) consisted of LC-10ADvp pump, SPD-10Av detector, CTO-10Avp column oven, SIL-10Dvp autosampler. The chromatographic separation was performed on a Shim-Pack VP-ODS column (4.6 mm 250 mm, 5 μm) which was held at 30°C. The optimized mobile phase was the mixture of citric acid-hydrochloric acid-sodium hydrate buffer (pH 3.0): acetonitrile: methanol (32:30:38, v/v/v) at a constant flow rate of 1.0 mL/min. The detector was set at 215 nm and the volume of the sample solution injected was 20 μL. Quantitation was based on peak area integrated by Shimadzu LC solution (Version 1.24).

Stock solution

Portions of 14.7 mg of triacetin, 179.8 mg of acetic ether, 69.8 mg of butyl acetate and 70.2 mg of amorolfine hydrochloride reference standards were transferred into 25 mL volumetric flask, shaken until dissolved and then diluted to volume with methanol to obtain a combined stock solution at 0.585 mg/mL of triacetin, 7.185 mg/mL of acetic ether, 2.784 mg/mL of butyl acetate and 2.516 mg/mL of amorolfine. The stock solution was filtered through 0.22 μm membrane filter and stored at 4°C.

Working standard solution

An aliquot of 1 mL of stock solution was transferred to a 10 mL volumetric flask and the volume was adjusted with mobile phase to obtain a solution at 58.51 μg/mL of triacetin, 718.5 μg/mL of acetic ether, 278.4 μg/mL of butyl acetate and 251.6 μg/mL of amorolfine.

Sample solution for liniment assay

The commercial available amorolfine hydrochloride liniment was evaluated. Due to the high viscosity of liniment, weighing method was used instead of conventional volume method used for liquid assay. For calculation of amorolfine percentage, specific gravity of the liniment was determined according to USP Pharmacopoeia.

The liniment was frozen for 30 min at refrigerator (4°C) before weighed to prevent the evaporation of organic solvent. Five samples were weighed separately and analyzed.0.125 g of the liniment was accurately weighed and transferred into 25 mL volumetric flask, shaken until dissolved and then diluted to volume with the mobile phase. The resultant solution was filtered through 0.22 μm membrane filter before injection

Method validation

The method validation was performed according to USP Pharmacopoeia. The final optimized concentration of the mobile phase was constructed based on the separation among four components and their peak parameters (capacity factor and tailing factor).

Specificity

In order to determine the specificity of the method, identification of four analytes was studied, comparing raw material with the corresponding standard reference. In addition, the retention time of each substance in the sample solution is identical to the retention time received by the standard solution. Another study was carried out to check the absence of interference by the blank excipients (ethanol and acrylic resin), which take part in the pharmaceutical formulation.

Linearity

Linearity solutions were prepared at five concentration levels from 60% to 140% of working concentration of four components by diluting stock solution with mobile phase. Calibration curves for concentration versus peak area were plotted for each compound and the obtained data were subjected to regression analysis using the least squares method.

Precision

The precision test was carried out by the intra-day and inter-day variability for triacetin, acetic ether, butyl acetate and amorolfine. The intra-day variability was determined by analysis of replicate (n=6) samples of three different concentrations on the same day and inter-day variability was determined by analysis of replicate (n=5) samples of three different concentrations on five consecutive days. Between runs, solutions were stored at 4°C. Triacetin, acetic ether, butyl acetate and amorolfine concentrations were determined and the relative standard deviations (RSD) were calculated to represent precision.

Accuracy

The accuracy of the method was determined as described in sample solution for liniment assay, by analyzing a series of blank excipients spiked with various concentrations of standard triacetin, acetic ether, butyl

acetate and amorolfine at 80% (n=3), 100% (n=3) and 120% (n=3) of the prescription amount. Each set of additions was repeated five times. The accuracy of the method was calculated by the quantity of triacetin, acetic ether, butyl acetate and amorolfine recovered in relation to the added amount.

Limit of detection and limit of quantitation

The LOD and LOQ for triacetin, acetic ether, butyl acetate and amorolfine were estimated at a S/N of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentrations.

Stability of analytical solutions

It is always important to get information about the stability of analytes in solutions, considering the necessity for analyzing large amount of samples or unexpected delay during analysis. In this study, the stability of triacetin, acetic ether, butyl acetate and amorolfine in sample solution and the mixed working standard solution was studied.

Sample and standard solutions were analyzed immediately after storage at ambient temperature (around 25°C) for 0, 2, 6, 9 and 24 h, respectively. The response of the four substances was not significantly altered over this period; the RSD (%) values of triacetin, acetic ether, butyl acetate and amorolfine were 1.71, 0.17, 1.08 and 0.54 in standard solution and were 1.03, 0.14, 0.69 and 0.15 in sample solution, respectively.

Robustness

To determine the robustness of the proposed method, experimental conditions were deliberately altered and the resolution between triacetin and acetic ether, acetic ether and butyl acetate, butyl acetate and amorolfine was evaluated.

To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mL/min. The effect of column temperature on resolution was studied at 25°C, 30°C and 35°C. The effect of pH on resolution was also studied by varying the pH from 2.8 to 3.2. In all the above conditions, the components of the mobile phase were kept constant.

RESULTS

System suitability

The chromatographic separation was performed on a C18 column (Shimadzu ODS, 250mm×4.6mm, 5μm particle size). The optimized mobile phase for separation of all four compounds is the mixture of citric acid-hydrochloric acid-sodium hydrate buffer, acetonitrile and methanol (32:30:38, v/v/v). The detection was conducted using UV-vis detector set at 215 nm. The chromatographs of the solutions were recorded using a constant flow rate of 1.0 mL/min. The method separated triacetin (t_R=3.8 min), acetic ether (t_R=4.1min), butyl acetate (t_R=6.7 min) and amorolfine (t_R=7.8 min) in less than 10 min with good resolution. Peak shapes and parameters are shown in fig. 1 and table 1.

Method validation

Specificity

The chromatogram of the standards of triacetin, acetic ether, butyl acetate and amorolfine (fig. 1a) indicated the adequate resolution of all the studied compounds. The chromatograms obtained with the blank excipients (fig. 1b) and the sample solution (fig. 1c) showed no interfering peaks at the same retention time of triacetin, acetic ether, butyl acetate and amorolfine.

Linearity

Linear calibration plots for triacetin, acetic ether, butyl acetate and amorolfine were obtained over the calibration ranges tested. The correlation coefficients, concentration range and the regression equations are presented in table 2. The correlation coefficients obtained were greater than 0.999. The results show that an excellent correlation between the peak area and concentration for all four components.

Precision

The data of intra-day and inter-day precision determined by three different concentrations as above can be seen from table 3. The RSD values were all less than 2%, assuring the precision of the method.

Accuracy

The results obtained for the accuracy study (recovery method) from 9 samples studied (n=3 for 80%, 100% and 120%) are presented in table 4 for the four components. Therefore, it can be concluded that the procedure provided acceptable accuracy for all the analytes in the model mixture.

Robustness

In all the deliberately varied chromatographic conditions (flow rate, column temperature and pH), the resolution among the four components and the selected factors remained unaffected, illustrating adequate robustness of the method.

Stability of analytical solutions

No significant degradation was observed within the indicated period, suggesting that both solutions were stable for at least 24 h, which was sufficient for the whole analytical process.

Limits of detection (LOD) and quantitation (LOQ)

The limits of detection (LOD) were found to be 5.88 ng/mL for triacetin, 0.44 ng/mL for acetic ether, 0.40 ng/mL for butyl acetate and 0.08 ng/mL for amorolfine.

Table 1: System suitability for analysis of triacetin, acetic ether, butyl acetate and amorolfine by the proposed HPLC method

Compound	Retention time (t_R, min)	Resolution	Capacity factor	Tailing factor
Triacetin	3.80	2.90	1.24	1.24
Acetic ether	4.12	1.61	1.30	1.48
Butyl acetate	6.72	9.18	1.21	1.32
Amorolfine	7.85	2.73	2.06	2.95

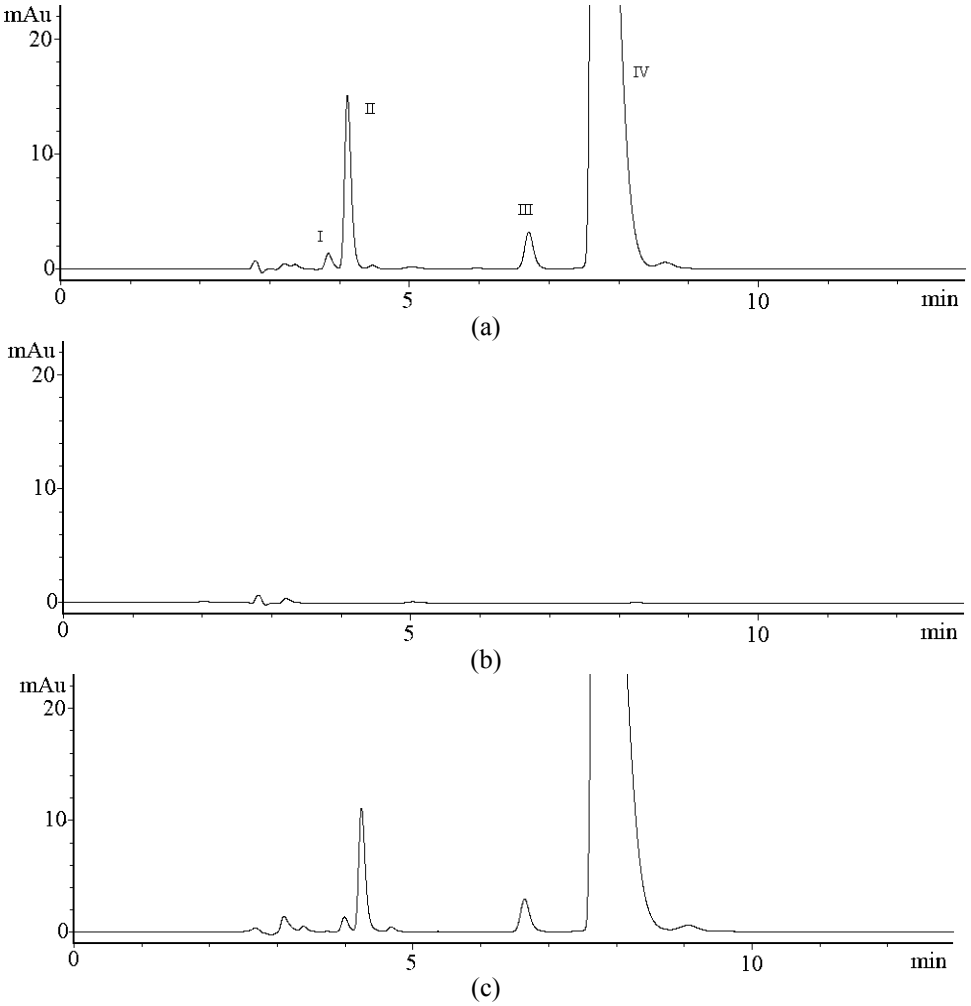

Fig. 1: HPLC chromatograms of standard solution of triacetin (I), acetic ether (II), butyl acetate (III) and amorolfine (IV) (a), blank excipients (b) and liniment sample (c).

The limits of quantification (LOQ) were found to be 17.7 ng/mL for triacetin, 1.47 ng/mL for acetic ether, 1.33 ng/mL for butyl acetate and 0.25 ng/mL for amorolfine.

Application: Assay of pharmaceutical liniment

The results for triacetin, acetic ether and butyl acetate, expressed as the weight in 100 grams of liniment, and the results for amorolfine, expressed as percentage drug recovery related to label claim, are presented in table 5. These indicate that amorolfine hydrochloride liniment uses 0.1009±0.00077 (%) as the plasticizer and the combination of acetic ether (1.4915±0.0040, %) and butyl acetate (0.5102±0.0021, %) as the organic solvents.

DISCUSSION

The chromatographic had good system suitability. Four components can be separated in less than 10 min with good resolution. Specificity, linearity, precision, accuracy and robustness were demonstrated by method validation. The stability of analytical solutions was sufficient for the whole analytical process. Using the established method,

Table 2: Linear regression data for analysis of triacetin, acetic ether, butyl acetate and amorolfine by the proposed HPLC method

Compound	Equations [a]	Concentration range (μg/mL)	Correlation coefficient(R^2)
Triacetin	Y=0.00479*X-0.601224	35.1-81.9	0.9997
Acetic ether	Y=0.007*X-5.796472	431.1-1005.9	0.9999
Butyl acetate	Y=0.00935*X-0.183046	167.0-389.7	0.9999
Amorolfine	Y=0.00015*X-0.717093	151.0-352.3	0.9999

a: X = peak area, Y= concentration of compound (μg/mL).

Table 3: Intra-day and inter-day precision of triacetin, acetic ether, butyl acetate and amorolfine by the proposed HPLC method

Compound	Concentration (μg/mL)	Inter-day	Intra-day
		RSD(%)[a]	RSD(%)[a]
Triacetin	44.67	0.96	1.86
acetic ether	55.84	1.01	1.92
butyl acetate	67.00	0.54	1.84
Acetic ether	597.1	0.36	1.42
butyl acetate	746.4	0.72	0.17
amorolfine	895.7	1.21	0.87
Butyl acetate	204.8	0.89	1.23
	256.0	1.22	1.08
	307.2	0.92	1.32
Amorolfine	200.1	0.53	1.12
	250.1	0.34	0.54
	300.1	0.67	0.89

[a] RSD (%) =(SD of amount detected/mean of amount detected)×100

Table 4: Accuracy study of the determination of triacetin, acetic ether, butyl acetate and amorolfine in model mixture by the chromatographic system (n=3)[a]

Spiked amount (%)	Triacetin		Acetic ether		Butyl acetate		Amorolfine	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
80	101.3	0.38	99.64	0.59	101.7	0.71	100.7	0.78
100	99.43	0.42	101.5	1.07	101.4	1.01	100.8	0.69
120	98.33	0.79	100.0	0.48	99.75	0.62	101.2	1.01

[a] All results are the average of three samples and are expressed as a percentage of the analytes added

Table 5: Assay of triacetin, acetic ether, butyl acetate and amorolfine in commercial amorolfine hydrochloride liniment (Batch 8212156) by the proposed HPLC method

Sample No.	Triacetin (%, g/g)[a]	Acetic ether (%, g/ml)[a]	Butyl acetate (%, g/ml)[a]	Amorolfine (%)[b]
1	0.1012	1.4887	0.5069	100.2
2	0.1005	1.4897	0.5115	99.65
3	0.1018	1.4911	0.5098	100.5
4	0.1012	1.4895	0.5105	100.9
5	0.0998	1.4985	0.5123	99.68
Mean	0.1009	1.4915	0.5102	100.2
RSD	0.76	0.27	0.41	0.54

[a] Expressed as the weight in 100 grams of liniment. [b] Percentage of drug recovered, relative to the label claim.

the amount of amorolfine in the liniment was determined to be within the USP requirements of 90-110% of the label claims.

CONCLUSION

The proposed isocratic RP-HPLC method for the simultaneous quantification of amorolfine, triacetin,

acetic ether and butyl acetate in marketed amorolfine liniment was shown to be reliable, simple, accurate, sensitive, precise and robust. The method was fully validated, showing satisfactory results for all the parameters tested. Moreover, it is fast and feasible. It should be practicably advantageous and can be considered for the determination of all four components in commercial liniment in the presence of ethanol and resin.

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