International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 9, Issue 5, 2017

Original Article

RAPID ISOLATION OF THE BIOACTIVE METABOLITE OF *CYMBOPOGON PROXIMUS* AND DEVELOPMENT OF LC METHOD

OLA MOHAMED EL-HOUSSINI[1], GHADA FAROUK METWALLY[2]*

[1]Department of Analytical Chemistry, [2]Department of Medicinal Plants and Natural Products, National Organization for Drug Control and Research (NODCAR), Agouza, Giza, Egypt
Email: ghadafarouk70@hotmail.com

Received: 16 Nov 2016 Revised and Accepted: 20 Mar 2017

ABSTRACT

Objective: Development of a rapid isolation method to afford the bioactive metabolite (proximadiol: prox) from its herb (*Cymbopogan proximus* Stapf.) in the purest form and validate an LC method for its determination was our goal in this work.

Methods: Prox was isolated by chromatographic techniques from the dichloromethane extract of the herb on alumina column, using petroleum ether-ethyl acetate solvent mixtures with increasing polarity.

The determination of prox was achieved by LC method. The chromatographic separation was carried out on Thermo Hypersil ODS (4.5 x 250 mm, 10 µm) column, in the presence of 8-chlorotheophylline as an internal standard. The mobile phase was composed of 0.1 M phosphate buffer (pH: 3.5): methanol (60:40 v/v) and was pumped at a flow rate of 1 ml/min. The detection and quantification were done at 210 nm.

Results: The structure elucidation of the isolated compound was identified on the basis of its spectral data.

A simple and reproducible LC method was developed for the determination of the isolated purified Prox. Adequate separation and good resolution were obtained between prox and 8-chlorotheophylline. Quantification was achieved at 210 nm over concentration range 12.00-33.60 µg/ml with mean percentage recovery of 99.29±0.340. The method was validated in accordance with USP specifications. All analytical criterions were within acceptable range. The validity of the results was assessed by applying standard addition technique. The results obtained were compared with the reported method.

Conclusion: The proposed method could be applied for routine quality control analysis of pharmaceutical formulations containing prox.

Keywords: *Cymbopogan proximus*, Identification, Isolation, LC method, Proximadiol (prox), Validation

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i5.16954

INTRODUCTION

Since the dawn of time, mankind has inherited the traditional use of medicinal plants in relieving pains and curing diseases. Despite their side effects, they persist as traditional medicine in developing countries [1]. Moreover, medicinal plants are considered as one of the most important sources that develop new drugs. A variety of herbal remedies has established therapeutic effectiveness. Such remedies are suitable for administration and are composed of either parts of a plant, simple extracts, or isolated bioactive metabolites. Most times the herbal products are introduced into the market with no suitable analytical evaluation. Consumers can buy such products without a physician's prescription and no one can determine the potential hazards in a lower grade product [2, 3].

Cymbopogan proximus Stapf. (Fig. 1) family Poaceae is one of the popular medicinal plants which are used for its effect as a renal antispasmodic and diuretic drug [4]. The pure plant extracts itself or admixed with synthetic chemicals are marketed in the Egyptian market for renal diseases. Some authors reported the sesquiterpene diol; proximadiol (prox) (fig. 2a) (Cryptomeridiol; (1R, 4aR, 7R, 8aR)-7- (2-hydroxypropan-2-yl)-1, 4a-dimethyl-decahydro-naphthalen-1-ol)) [5] to be the responsible metabolite for the antispasmodic activity [6, 7]. Published data was reported for isolation of this bioactive metabolite from the plant by using silica gel column chromatography of the petroleum ether extract [8], moreover, the same compound had also been isolated after saponification of the petroleum ether extract by subjecting the unsaponifiable matter to vacuum liquid chromatography (VLC) over silica gel then chromatographic separation of one of the fractions on alumina column [4].

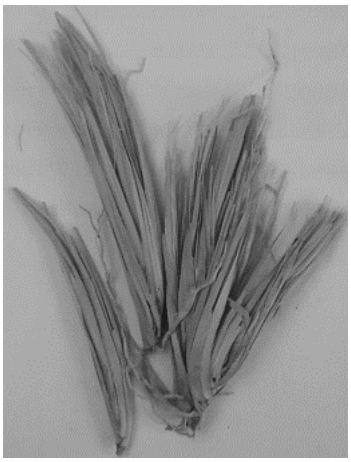

Fig. 1: *Cymbopogan proximus* herb

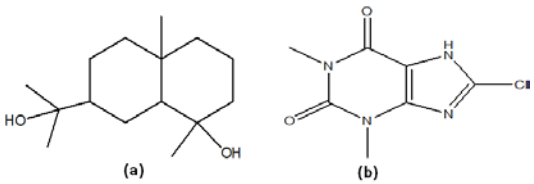

Fig. 2: Chemical structures of: (a) proximadiol (prox) and (b) 8-chlorotheophylline

Quality control of phytopharmaceuticals is essential to approve the efficacy and safety of the product. It is concerned with identity, purity and content, besides another chemical, physical and biological properties. Quality control methods of herbal drugs are sophisticated [3]. The quantification of prox had been previously performed using gas liquid chromatography (GLC) [8], UV spectrophotometry [9], thin layer chromatography (TLC) [8, 10] and UV assisted-principal component analysis (PCA) and partial least square (PLS) [8] methods. Nowadays, high-performance liquid chromatography (LC) is considered an efficient tool to evaluate herbal drugs due to its high sensitivity and reliability. None of the reported methods was LC. Thus, this work aims to promptly isolate and identify prox from the plant and then use it as a standard to assess the quality of its pharmaceutical formulations. As LC is still one of the most popular methods for herbal analysis [11], so, our goal was also to develop and validate a simple, economic, sensitive, specific, accurate, precise, and robust LC method for prox determination. The method is based on the separation of prox on RP column in presence of 8-chlorotheophylline as internal standard (fig. 2b). The proposed LC method has a distinctive advantage over the reported methods regarding simplicity, accuracy, precision and limit of detection.

MATERIALS AND METHODS

Materials

Plant material

The plant material was purchased from Harraz Herbal Drugstore in Cairo, Egypt in 2014 and was kindly identified by Dr. Reem Sameer (Ass. Prof. Botany department, Faculty of Science, Cairo University). A voucher specimen (Poa/Cp/25-9/14) was kept in the herbarium of the department of medicinal plants and natural products (NODCAR).

Chemicals and reagents

Prox was isolated and its purity was assessed as mentioned under (isolation and identification of prox). 8-chlorotheophylline certified to have a purity of 100%, was supplied from Adwic Egypt. Proximol compound® effervescent granules nominally containing 18.6 mg of Halfa bar (*Cymbopogan proximus*) extract equivalent to 8 mg prox in each 100 g, were purchased from Kahira Pharmaceuticals and Chemical Industries Company, Cairo, Egypt (B. No. 1540446). All solvents used were of LC grade and all reagents were of analytical grade. Methanol, dichloromethane, petroleum ether and ethyl acetate were purchased from Sigma-Aldrich (Germany), Fisher Chemical (UK), Scharlau (Spain) and CarloErba Reagents (France), respectively. Alumina (Al2O3 90) was obtained from Merck Dramstadt (Germany). Potassium dihydrogen orthophosphate and sodium hydroxide were obtained from Merck (Germany). O-phosphoric acid (85%) was purchased from VWR Chemicals (UK). Phosphate buffer (0.1M) was prepared by dissolving 6.8 g potassium dihydrogen orthophosphate in 450 ml water, adjusting pH to 3.5 with O-phosphoric acid and then diluted to 500 ml with water. Spraying reagent p-anisaldehyde-sulphuric acid was used for visualizing the isolated compound and prepared according to Wagner et al. [12].

Instrumentation

The purity of prox was checked by confirming the melting point (Electrothermal 9100, UK), UV spectrum (Unicam, UK), infrared spectrum (IR Prestige-21, Shimadzu, Japan), NMR spectrum (Bruker, Avance III, 400MHZ, 5 mm probe, Massachusetts, USA) and mass spectrum (UPLC MS. MS, Waters, USA). A rotary evaporator (RII, Buchi, Germany) was used for evaporating the solvents from the extract, under vacuum. TLC plates used were silica gel/TLC cards with fluorescent indicator 254 nm, layer thickness: 0.2 mm (20 x 20 cm aluminium cards) Fluka packed in Buchs, Switzerland. The chromatographic separation was achieved using an HPLC system (Agilent 1200, Germany), equipped with vacuum degasser, quaternary pump, column oven, UV/VIS detector, Thermo Hypersil ODS (4.5 x 250 mm, 10 µm USA) (SN 30110-254630) column and was supported by Agilent LC chemstation software for data acquisition. Other investigated columns were: Luna phenomenex C8 100A (4.5 x 250 mm, 5 µm USA) (SN H15-028607), Symmetry C18 (4.5 x 150 mm, 5 µm Ireland) (SN 028334104238 65). Hamilton 50 µl syringe (Sigma-Aldrich, Germany) was used for injection. All weightings were done on Sartorius CP224S balance (Germany). Elma sonic P60 H ultrasonic processor (Germany), pH-meter Jenway 3510 (UK) and water purification system Mili-QR Direct 8-16 system (France) were used. Membrane filters 0.45 µm from Teknokroma (Spain) were used for filtration of the mobile phase.

Isolation and identification of prox

The dried powdered herb (250 g) was exhaustively extracted with dichloromethane by the aid of sonication at room temperature. The collected extract was filtered and the solvent was evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C. The obtained residue (20 g, yellowish green fatty residue) was chromatographed on a glass column of 60 g of alumina). Petroleum ether (40-60 °C) was used for elution and the polarity was raised by increasing concentrations of ethyl acetate (up to 80%) and 100 ml fractions were collected. Similar fractions were pooled together after screening with TLC using S_1 (dichloromethane: methanol, 9:1) as the mobile phase. Fraction 19-22 (200 mg) was rich in prox. The compound was purified by recrystallization from a methanolic solution by using petroleum ether. Identification of prox was based on checking its melting point, UV, IR, ESI/MS and ^{1}H-NMR spectra and comparing these data with the published ones [13, 14].

Standard solutions

Preparation of prox stock solution

Accurately weighed 48 mg of prox was transferred into a 50-ml volumetric flask and dissolved in 20 ml methanol. The volume was then completed to mark with the same solvent. (0.96 mg/ml)

Preparation of prox working solution

From the previous stock solution, 5 ml was transferred into a 50-ml volumetric flask and dissolved in 20 ml methanol. The volume is completed to mark with the same solvent. (0.096 mg/ml)

Preparation of internal standard stock solution

Accurately weighed 25 mg of 8-chlorotheophylline was transferred into a 100-ml volumetric flask and dissolved in 80 ml methanol. The volume was completed to mark with the same solvent. (0.25 mg/ml)

Chromatographic conditions

Chromatographic separation was carried out on thermo hypersil ODS C_{18} column (250 x 4.6 mm, 10 µm USA), using mobile phase consisting of 0.1 M phosphate buffer (pH 3.5): methanol (60:40 v/v) in presence of 8-chlorotheophylline as the internal standard. The detection was done at 210 nm. The mobile phase was pumped at a flow rate of 1 ml/min. Separation was done at 25 °C and the run time was 6.5 min. The LC system and column were equilibrated and saturated with mobile phase for 45 min before injection of samples. Data acquisition was performed on Agilent LC chem station software.

Sample preparation

The content of 5 bottles of the proximol compound® effervescent granules was emptied and well mixed. An amount of 100 g of the granules was accurately weighed and then transferred into a 500-ml beaker. A suitable amount of purified water was added. The beaker was left until the effervescence ceased. The solution was then transferred into a 250-ml separating funnel. Exhaustive extraction was made with dichloromethane (3 x 50 ml). The collected extracts were collected and evaporated under vacuum. The dried residue was dissolved into methanol and transferred to the 100-ml volumetric flask to afford a solution of 0.08 mg/ml.

Procedures

Construction of calibration curve

Accurately measured volumes of prox, equivalent to 0.120–0.336 mg from its working solution with 0.05 mg 8-chlorotheophylline from its stock solution, were transferred into a series of 10-ml volumetric flasks and the solutions were completed to mark with methanol. A volume of 20 µl of each solution was injected in triplicates using Hamilton 50 µl syringe. A calibration curve was obtained by plotting the area under the peak versus the drug concentration (12.00-33.60 µg/ml) for prox and regression equation was computed.

Assay of sample

The procedure described under construction of calibration curve was repeated using volumes of prox equivalent to 0.120–0.336 mg with 0.05 mg 8-chlorotheophylline. The same conditions were used to determine prox in the sample. The concentration of prox was determined by the use of its regression equation.

RESULTS AND DISCUSSION

Isolation, purification, and characterization of prox

The method of isolation used in this study was briefer than those previously reported [4, 8, 13, 14]. From the economic point of view, this method saved much time and solvents compared to previous methods; additionally it produced the required compound in a good yield. Meanwhile, ultrasonic assisted extraction method provides higher recovery of bioactive compounds and led to lower consumption of solvent [15]. Moreover, using dichloromethane ensured better extraction of the desired compound than using petroleum ether in its extraction. Another advantage in such method was the inclusion of alumina instead of silica gel in column chromatography, which was more advantageous due to its stability at different temperatures, pH values and did not break down like silica [16].

The isolated compound (100 mg), prox, was afforded as white amorphous powdered solid. It was highly soluble in dichloromethane and methanol, but less soluble in petroleum ether. It showed a single spot in TLC with solvent system S_1 (R_f: 0.57) that gave bluish violet color after spraying with the p-anisaldehyde-sulphuric acid reagent. Its melting point was found to be between 137-142 °C. It showed the following spectral characteristics: UV: λ_{max} cm^{-1} 395 in dichloromethane. IR (KBr) *v* max cm^{-1}: 3429, 2968, 1465, 1382, and 1178. The IR spectrum revealed the presence of strong broad hydroxyl absorption (3429 cm^{-1}) and no bands detecting the presence of unsaturation in the compound. ESI/MS (*m/z*): 503 (36%) for $[2M+Na]^+$and 263 (100%) for $[M+Na]^+$, these results proved that the molecular formula of the isolated compound was $C_{15}H_{28}O_2$. ^{1}H-NMR (400 MHz, DMSO): δ = 0.79 (s, 3H, CH_3-14), 0.85 (br. s, 3H, CH_3-15), 0.95 (s, 0.95, 6H, CH_3-12 and CH_3-13), 1.05 (d, j=8, 1H, H-1ax), 1.09 (br s, 1H, H-6ax), 1.17-1.19 (m, 1H, H-5), 1.28 (br s, 1H, H-7), 1.31-1.33 (m, 1H, H-1eq), 1.36 (br s, 1H, H-3ax), 1.43-1.45 (m, 1H, H-2), 1.62 (br s, 1H, H-3eq), 1.88-1.91 (m, 1H, H-6eq). The ^{1}HNMR spectrum proved the presence of the methyl groups (δ= 0.79, 0.85 and 0.95), in addition to the two methine protons (δ= 1.18 and 1.28 for H-5 and H-7, respectively).

Based on the physical properties, TLC and by comparing the obtained spectral data with the published ones [13, 14], the structure of the compound was elucidated as prox.

LC method

By reviewing literature, no LC method had been reported for the determination of prox in pure form or in pharmaceutical preparations. Hence, the present work was concerned with the development and validation of simple, sensitive LC method for the determination of prox in pure form and in its pharmaceutical dosage form.

Assay parameters

The chromatographic separation was performed on Thermo Hypersil ODS C_{18} column (250 x 4.6 mm, 10 µm), using mobile phase composed of 0.1 M phosphate buffer (pH 3.5): methanol (60:40 v/v) in presence of 0.05 mg 8-chlorotheophylline as internal standard at 25 °C. Flow rate of 1 ml/min was chosen. The detection based on peak area was conducted at 210 nm, where the linear correlation was obtained between peak area and concentration (fig. 3). System suitability parameters were studied to verify the system performance. Results were shown in tables 1, 2.

Table 1: Validation data for the analysis of prox by the proposed LC method

LC method	Parameters		
Linearity (µg/ml)	12.00-33.60		
LOQ (µg/ml)	0.768		
LOD (µg/ml)	0.230		
t_R Prox(min)	2.72		
Accuracy	99.29±0.340		
Precision			
Concentrations (µg/ml)	19.20	24.00	28.80
Repeatability	0.117	0.063	0.132
Intermediate precision	0.255	0.603	0.408
Regression equation	0.082x+0.047		
Slope	0.082		
SE of slope	$5.550x10^{-3}$		
Sb	$1.241x10^{-3}$		
Confidence limit of slope	$0.082±3.949 x10^{-3}$		
Intercept	0.047		
SE of intercept	0.013		
Sa	0.0289		
Confidence limit of intercept	0.047±0.092		
Correlation coefficient	0.9995		
SE of estimation	0.0208		

LOQ: limit of quantification; LOD: limit of detection; t_R: retention time; RSD: relative standard deviation; SE: standard error; Sb: standard deviation of slope; Sa: standard deviation of intercept; accuracy (n=5, results: mean±RSD); repeatability (n=3x3 different concentrations, results: % RSD); intermediate precision (n=9x3 different concentrations, results: % RSD).

Table 2: Test results of system suitability for the proposed LC method

Parameters	prox
N	764
T	0.89
R	6.76
α	12.85
K'	1.33

N: no of theoretical plates per column, T: tailing factor, R: resolution factor, α: selectivity factor, K': capacity factor.

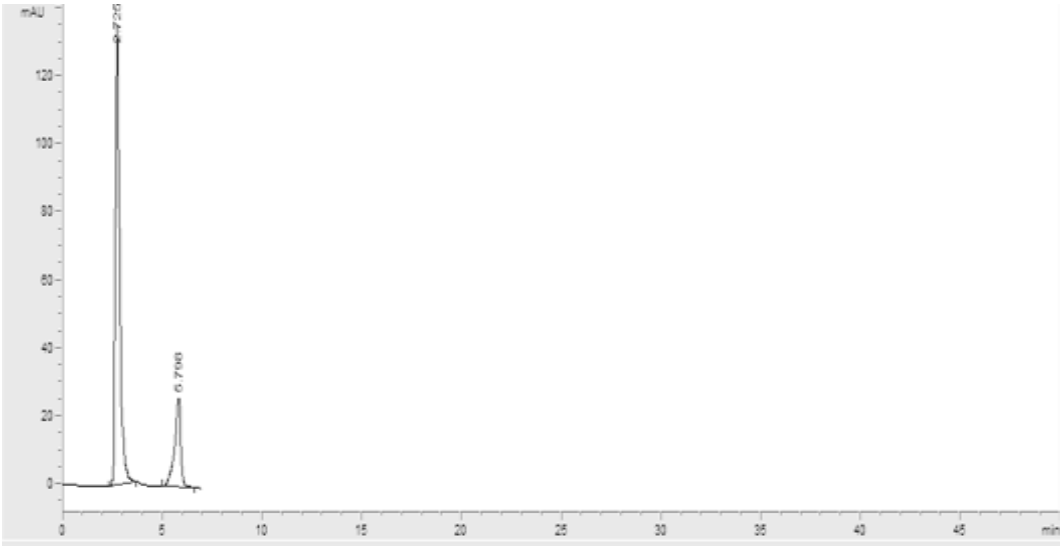

Fig. 3: A typical LC chromatogram of prox (t_R 2.73 min) and 8-chlorotheophylline (t_R 5.79 min)

All parameters affecting the chromatographic separation were studied. Several trials were done to separate prox using different mobile phases and number of columns. The chromatographic separation was achieved using mobile phase composed of 0.1 M phosphate buffer (pH 3.5): methanol (60:40 v/v). The composition of mobile phase was carefully adjusted by varying the organic modifier to aqueous ratio and also by altering the pH and ionic strength of buffer to give optimum results. Increasing the proportion of methanol by 80% prolonged the retention time of prox and led to peak broadening, as by decreasing the polarity of solvent-polar solute tended to spend more time on the stationary phase. While increasing methanol by 20%, did not affect much resolution. Increasing the ionic strength of the buffer, while decreasing its pH peak tailing tended to decrease and the smooth baseline was obtained. As working with high ionic strength buffer at low pH values masked the effect of the residual silanol groups on RP column by protonating them. Among the entire investigated columns Thermo Hypersil ODS C_{18} column (250 x 4.6 mm, 10 µm) showed the best resolution, peak shape and smooth baseline. It was the column of choice due to its high number of theoretical plates, rapid equilibrium and end capping. It also had the advantage of its low cost. Methanol was favoured as a solvent because it was a good solvent for Prox and its low cost in comparison with acetonitrile. The internal standard was used to correct small variation within the analytical process and to improve accuracy and precision of the method. 8-chlorotheophylline was chosen due to its stability.

Besides, it was well resolved from Prox, did not react with the test material and it did not contain impurities with the same retention time of Prox. The flow rate of 1 ml/min was found to be suitable for good resolution between Prox and 8-chlorotheophylline within a rational time. Several wavelengths (210, 220 and 254 nm) were tried for detection and quantification. The detection and quantification were carried out at 210 nm as prox gave a high response and it was away from the UV cut-off of methanol (205 nm). Changing column temperature (20, 25, 30 °C) did not affect resolution. So detection was performed at ambient temperature (25 °C).

Analysis of sample

The proposed method was applied to determine prox in Proximal compound® effervescent granules. Satisfactory results obtained for prox were in good agreement with the label claimed (table 3). The typical chromatogram was obtained that shows no interferences from the excipients and additives (fig. 4).

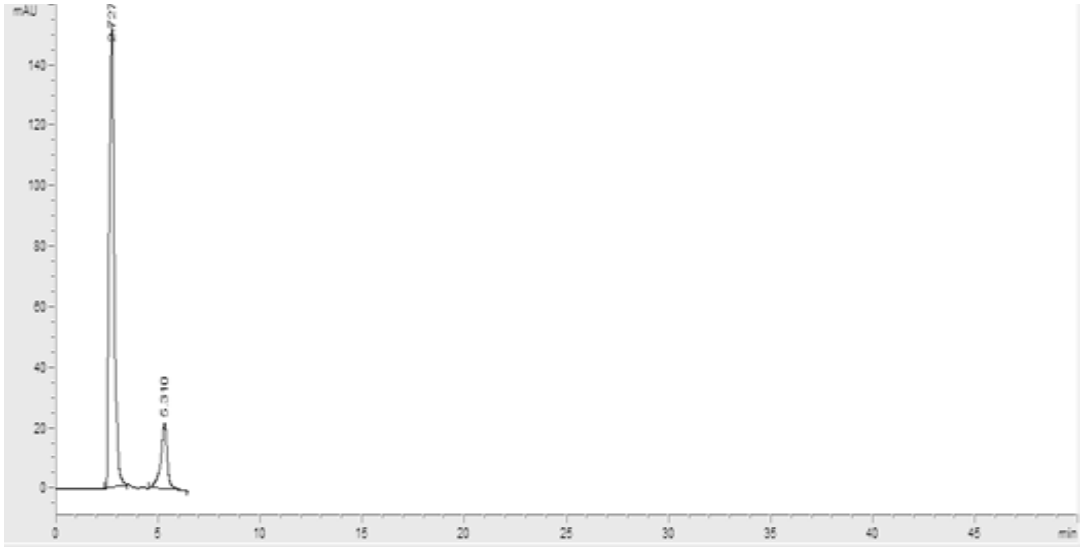

Fig. 4: A typical LC chromatogram of sample solution of prox (t_R 2.73 min) and 8-chlorotheophylline (t_R 5.31 min)

Table 3: Application of the proposed LC method for the determination of prox in Proximol compound® effervescent granules (8 mg/100 g)

Proximol compound® (8 mg/100 g)	LC method
Recovery*	93.28±0.889
Standard Addition*	99.38±0.165

*n =3x3 different concentrations, results: mean±RSD

System suitability

System suitability tests are a fundamental part of LC methods. These tests are used to confirm that the chromatographic system is adequate for the proposed analysis [17]. System suitability parameters are calculated according to USP [17] and it is found to be satisfactory (table 2).

Number of theoretical plates (column efficiency) (N)

Column efficiency reflects the performance of the stationary phase. N depends on the compound chromatographed, operating conditions including flow rate, the temperature of the mobile phase, quality and uniformity of packing within the column [17]. The more theoretical plates available within a column, the more equilibriums are possible and better quality of separation. The N value is presented in table 2.

Tailing factor (T)

Tailing factor also known as symmetry factor of a peak reflects peak asymmetry. The ideal chromatographic peak should be symmetrical (Guassian). However, due to factors such as dead volume, adsorption effect of the stationary phase and column packing, many chromatographic peaks do not appear in this ideal shape. Asymmetric peaks are difficult to resolve, so their integration to produce peak area for quantification are less reproducible. The acceptable value of T is obtained and it is represented in table 2.

Resolution (R)

Resolution is the measure of the extent of separation between two adjacent peaks [18]. A resolution value of 1.5 implies that the sample components are well separated to the extent that the peak area of each component is accurately measured. In the present work, R is more than 1.5 and it is presented in table 2.

Selectivity factor (α)

Selectivity factor also known as separation factor is the ability of the chromatographic system to distinguish between two components within the same sample. High values represent good separation. Results are presented in table 2.

Capacity factor (K')

Capacity factor also known as retention factor is the measure of the retention time of a compound in the sample with a given combination of mobile phase and column. For an optimum separation, retention factor should be in the range of 0.5< K'<10 [18]. A satisfactory result is presented in table 2.

Method validation

Method validation is used to prove that the method applied for a specific test is suitable for its proposed use. Validation of the proposed method is conducted according to the USP [17] recommendations.

Linearity and range

A linear relationship is obtained between the area under the peak and the concentration of prox in the range of 12.00-33.60 µg/ml. The linearity is validated by high values of r and small values of the standard error of estimation, intercept and slope as shown in table 1.

Accuracy

Accuracy of the experimental results is calculated from five samples of prox analyzed by the proposed method (each concentration is the average of three experiments) as presented in table 1. The results obtained are compared with the reported TLC method (mobile phase: chloroform: methanol (95:5, v/v) that used percolated silica gel plates 60 F_{254}(10 x 10 cm, 0.2 mm thickness) and sprayed the plates with spraying reagent after developing, then heated to 160 °C for three minutes) [10]. The proposed method shows high accuracy and precision in comparison with the reported TLC method [10]. Standard addition technique is applied to further proof the accuracy of the proposed method. The mean percentage recovery and RSD values are shown in table 3.

Precision

Repeatability and intermediate precision are performed as an evidence of precision**.**

Repeatability

The repeatability is evaluated through replicate analysis of prox by the proposed method. Each concentration is analysed three times. The mean percentage recoveries and RSD are shown in table 1. Small values of RSD indicate the high precision of the proposed method.

Intermediate precision

The intermediate precision is evaluated through replicate analysis of prox by the proposed method on three successive days. The percentage recoveries based on average of three separate determinations are shown in table 1. Small values of RSD indicate the high precision of the proposed method.

Table 4: Results of robustness for the proposed LC method

Parameters	Conditions	R
Mobile phase composition	59:41	6.89
Buffer: Methanol	60:40	6.76
60:40	61:39	6.00
		RSD=0.056
Temperature [°C]	23	6.67
	25	6.76
	27	6.67
		RSD=0.006
Flow rate [ml/min]	0.9	7.20
	1	6.76
	1.1	6.84
		RSD=0.027
pH of buffer	3.4	6.56
	3.5	6.76
	3.6	6.67
		RSD=0.010

Specificity

Specificity is the ability of an analytical method to measure analyte response in the presence of interferences. Specificity is checked by analyzing prox in Proximol compound® effervescent granules. The chromatogram of the pharmaceutical formulation shows good resolution without any chromatographic interference from excipients and no extra peaks (fig. 4). This proves the ability of the method to determine prox in sample solution without the interference of excipients (table 3).

Limits of quantification and limit of detection

The limit of quantification (LOQ) is the concentration of the analyte at which the signal-to-noise ratio (S/N) is ten and the limit of detection (LOD) at which the signal-to-noise ratio is three. The obtained values are determined experimentally, which are the averages from six replicates (table 1).

Robustness

The robustness of the proposed procedure is proved by its ability to resist any change in chromatographic conditions. Changing the aqueous to the organic proportion (±1%), pH of buffer (±0.1), the flow rate of mobile phase (±0.1 min) and column temperature (±2 °C) do not affect resolution, indicating the method to be robust (table 4).

CONCLUSION

The present work demonstrates the isolation of prox from the herb of *Cymbopogan proximus* in a simpler and more rapid method than those previously reported methods. Moreover, the proposed LC method is considered the first validated LC method for prox determination. It represents a simple, sensitive, economic, accurate and specific method for the determination of prox isolated from the dried plant and in pharmaceutical formulations without interferences from excipients. The method shows satisfactory results for all validation parameters tested. By comparing the proposed method with other reported methods, it is found to be more accurate and precise. So, it is considered as the method of choice for determination of prox in quality control laboratories.

AUTHORS' CONTRIBUTION

GFM contributed in collecting the plant sample and identification, running the laboratory work concerning the isolation of the compound and analysis of the spectral data. OME contributed to the laboratory work dealing with the development and validation of the LC method. Both authors designed the study, contributed to critical reading of the manuscript and approved the submission.

CONFLICT OF INTERESTS

The authors have no conflict of interest to disclose.

REFERENCES

1. Ennacerie FZ, Rhazi FF, Rahou A. Ethnobotanical study of medicinal plants used in traditional medicine in the province of Sidi Kacem, Morocco. Asian J Pharm Clin Res 2017;10:121-30.
2. Van der Kooy F, Federica Maltese F, Choi YH, Kim HK, Verpoorte R. Quality control of herbal material and phytopharmaceuticals with MS and NMR-based metabolic fingerprinting. Planta Med 2009;75:763-75.
3. Ahmad I, Aqil F, Owais M. Modern phytomedicine. Turning medicinal plants into drugs. Weinheim; WILEY-VCH Verlag GmbH and Co. KGaA; 2006.
4. El-askary HI, Meselhy MR, Galal AM. Sesquiterpenes from *Cymbopogon proximus*. Molecules 2003;8:670-7.
5. http://en.chembase.cn/molecule-305224.html. [Last accessed on 10 Oct 2016]
6. Locksley HD, Fayez MBE, Radwan AS, Chari VM, Cordell GA, Wagner H. Constituents of local plants: XXV, the constitution of the antispasmodic principle of *Cymbopogon Proximus*. Planta Med 1982;45:20-2.
7. Evans FE, Miller DW, Cairns T, Baddeley GV, Wenker E. Structure analysis of proximadiol (cryptomeridiol) by ^{13}CNMR spectroscopy. Phytochemistry 1982;21:937-8.
8. Abou-Shoer MI, Fathy HM, Omar AA. Extract-template modeling and pattern recognition in the assessment of *Cymbopogon proximus*. Am J Anal Chem 2011;2:500-10.
9. Radwan AS. An analytical method for proximadiol, the active principle of *Cymbopogon Proximus*. Planta Med 1975;27:93-7.
10. Temraz AA, El-naggar D. Development of TLC-densitometeric method used for quantitative estimation of some natural pharmaceutical preparations in the Egyptian market. J Am Sci 2012;8:367-71.
11. Atri P, Bhatti M, Kamboj A. Development and validation of rapid reverse phase high-performance liquid chromatography method for simultaneous estimation of stigmasterol and β-sitosterol in extracts of various parts (leaves, stems and roots) of *Xanthium strumarium* Linn. Asian J Pharm Clin Res 2017;10:234-8.
12. Wagner H, Bladt S, Zgainski EM. Plant drug analysis. Berlin, Heidelberg, New York, Tokyo: Springer-Verlag; 1984.
13. Kengne ABO, Tene M, Tchinda AT, Pierre Tane P, Frédérich M. Terpenoids from *Phaulopsis imbricata* (Acanthaceae). J Med Plants Res 2016;10:122-9.
14. Werner I, Mucaji P, Presser A, Glasl S. Sesquiterpenes and phenolic compounds from *Achillea clypeolata*. Z Naturforsch 2007;62b:267-71.
15. Santos HM, Lodeiro C, Capelo-Martinez JL. The power of ultrasound. In: Capelo-Martinez JL. Ed. Ultrasound in chemistry: Analytical applications. WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim; 2009.
16. http://www.dynamicadsorbents.com/aluminasuperior.htm [Last accessed on 10 Oct 2016]
17. The United States Pharmacopoeia (USP39), the United States Pharmacopoeial Convention, Rockville; 2016.
18. Hadad G. Validated stability-indicating HPLC method for the determination of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylene dioxybiphenyl-2,2'-dicarboxylate (DDB) and its degradation products. J Pharm Biomed Anal 2008;47:695-703.

How to cite this article

- Ola Mohamed El-Houssini, Ghada Farouk Metwally. Rapid isolation of the bioactive metabolite of *Cymbopogon proximus* and development of LC method. Int J Pharm Pharm Sci 2017;9(5):75-80.