Estimation of Costunolide and Dehydrocostus Lactone in *Saussurea lappa* and its Polyherbal Formulations followed by their Stability Studies Using HPLC-DAD

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ABSTRACT

Background: *Saussurea lappa* is one of the popular Ayurvedic herb; costunolide and dehydrocostus lactones are well-known sesquiterpene lactones contained in many plants used as popular herbs, such as *S. lappa,* and have been considered as potential candidates for the treatment of various types of tumor. **Objective:** The present study was used for the quantification of costunolide and dehydrocostus lactone in *S. lappa* and its polyherbal formulations, stability studies of markers and characterization of their degradants. **Materials and Methods:** HPLC analysis was performed on Waters NOVAPAK HR C_{18} column (300 mm × 3.9 mm i.d., 6 µm) using isocratic elution with acetonitrile and water (60:40% v/v). **Results:** The calibration curves of both analytes showed good linearity within the established range 5-100 µg/ml. The limits of detection (LOD) and quantification (LOQ) were 1.5 and 4.6 µg/ml for costunolide and 1.3 and 4.0 µg/ml for dehydrocostus lactone, respectively. Good results were achieved with respect to repeatability (%RSD < 2.0) and recovery (99.3-101.8%). **Conclusion:** The method was found to be precise, accurate, specific, and was successfully used for analyzing costunolide and dehydrocostus lactone in *S. lappa* and its polyherbal formulations. The developed method was found to be suitable for stability studies of markers and characterization of their degradation products.

Key words: Costunolide, dehydrocostus lactone, HPLC, stability studies

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INTRODUCTION

Herbal formulations for healthcare have largely been empirical with regards to their phytochemical profile, biopotency, toxicity, etc., Recent evidence on phytochemicals in chemoprevention[1] and as therapeutics[2] has promoted systematic investigation of traditional healthcare that includes standardization and identification of chemical markers in herbal drugs and clinical trials for validation of health claims. The phytochemicals in the plants are subjected to wide variations due to growing season,[3] maturity,[4] post-harvest processing[5] and variety.[6] In addition, the methods employed in traditional medicines for extraction and concentrations have a tremendous impact on phytochemical profiles of formulations. Standardization, in terms of quantitative profiling of active principles from plants to their end products (herbal formulations), including their stability, therefore, is vital to provide credence to health claims and for quality assurance.

Saussurea lappa Clarke (Compositae) is indigenous to India and Pakistan, where it grows in the Himalayas at 2500-3500 m elevations. Sesquiterpene lactones are the most common constituents of *S. lappa.*[7] Dried 4- to 5-year-old roots of this plant are known as costus roots and have reputation for their usage in traditional systems of India, China and Japan.[8,9] The effective and common components of this herb are sesquiterpene lactones. Costunolide (COS) and dehydrocostus lactone (DE COS) are the major components, whose chemical structures are given in Figure 1.[10,11] Pharmacological evaluation of these markers revealed that they not only have the effect of antimicrobial,[12-14] anti carcinogenic[15-17] and anti ulcer activities,[18,19] but also anti-inflammation[20] and protein tyrosine phosphate inhibitory activities.[21] This plant is being used widely in Ayurvedic herbal formulations. Quality of plant materials which are used in herbal

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formulations is essential for herbal formulation to be more active. Quality of plant material will be considered good only if the active constituents are present. The content of COS and DE COS had been used as the standard index to appraise the quality of *S. lappa* and its products. Consequently, the isolation, purification and determination of the markers, COS and DE COS become increasingly important. Therefore, few analytical[22,23] and bio-analytical methods[24-26] have been reported in the literature for the estimation of COS and DE COS. However, these methods were restricted to determination of these markers from *S. lappa* and were not studied for their polyherbal formulations. As part of pharmacological-phytochemical integrated studies of medicinal plants from Indian flora, we are investigating the chemical composition of *S. lappa* as well as their cytotoxic activity. In the course, we have recently reported sesquiterpenes from the *S. lappa* which displayed moderate cytotoxicity against human cancer cells[27] and the estimation of costunolide.[28] In the present study, high-performance liquid chromatography (HPLC) method was developed for separation and quantification of costunolide and dehydrocostus lactone and its marketed polyherbal formulations. In addition, we also studied the stability of these markers under various conditions. We herein report the simultaneous estimation of costunolide and dehydrocostus lactone from *S. lappa* and its polyherbal formulation along with the stability studies of the markers.

EXPERIMENTAL

Plant material, Chemicals and standards

Roots of *S. lappa* were obtained from the Himalayan region, Srinagar, JandK, India and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Thirupati. Voucher specimen of the plant material SL-NPL-13 of *S. lappa* was kept at Natural Products Laboratory of Indian Institute of Chemical Technology, Hyderabad. They were shade dried, fine powdered and used for analysis. Dried and powdered roots were extracted using different solvents, that is, chloroform, methanol and aqueous methanol. Column chromatography was carried out using silica gel 60-120 mesh (Qingdao Marine Chemical, China) and precoated silica gel plates (Merck, $60F_{254}$) were used for thin layer chromatography (TLC). Ethyl acetate, hexane and chloroform for isolation process were purchased from local distributor. HPLC grade acetonitrile and methanol used for the HPLC analysis were obtained from Merck India. Concentrated hydrochloride, anhydrous sodium hydroxide pellets, hydrogen peroxide (6% H_2O_2) of analytical grade were purchased from S.D. Fine chemicals, Hyderabad, India. Ultra pure water for chromatographic use was obtained from a Milli-Q system (Millipore Corp., Bedford, MA, USA). All the samples were filtered through the 0.45 μm membrane filter before injecting into HPLC. Two bioactive compounds, COS and DE COS were isolated from hexane extract in our laboratory as described earlier.[27] Their structures were identified by comparison of their spectral data (UV, IR, MS, ^{1}H NMR, ^{13}C NMR and 2D NMR) with those published in references. The purity of each compound was determined to be more than 98% by normalization of the peak area detected by HPLC. Chemical structure of the isolates was shown in [Figure 1]. Chromatographic separation was achieved using Waters NOVAPAK HR C_{18} column (300 × 3.9 mm i.d., 6 μm particle size).

Preparation of sample (Polyherbal formulations)

Various polyherbal formulations (tablets, powder and oil) were purchased from the market and were extracted using different procedures. For powder formulation 1.0 g was weighed. For tablets, average weight of 10 tablets was taken and finely powdered from which average weight of one

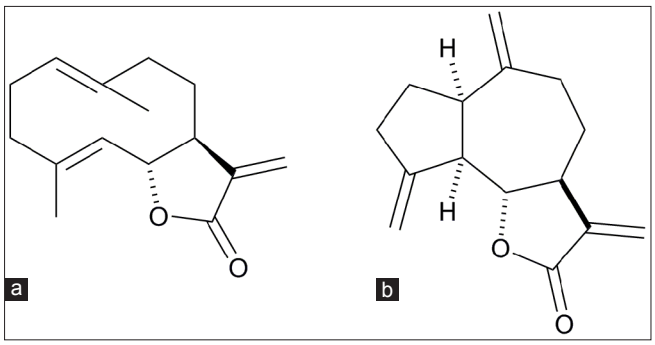

Figure 1: Chemical structures of (1) Costunolide (COS), (2) DP-1, (3) Dehydrocostus lactone (DE COS) and (4) DP-2

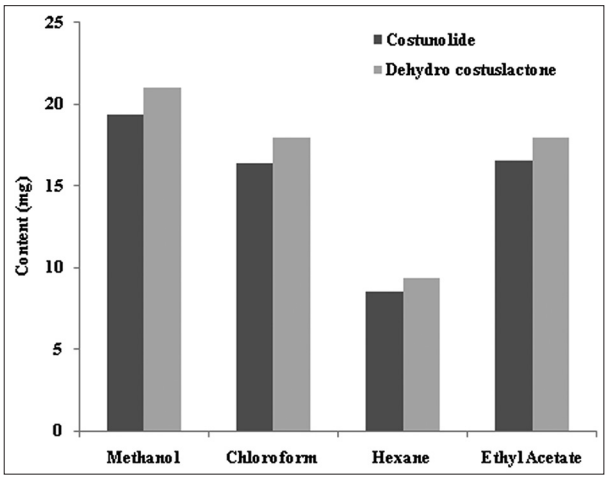

Figure 2: Effect of different solvents on the extractability of COS and DE COS from roots of *S. lappa*

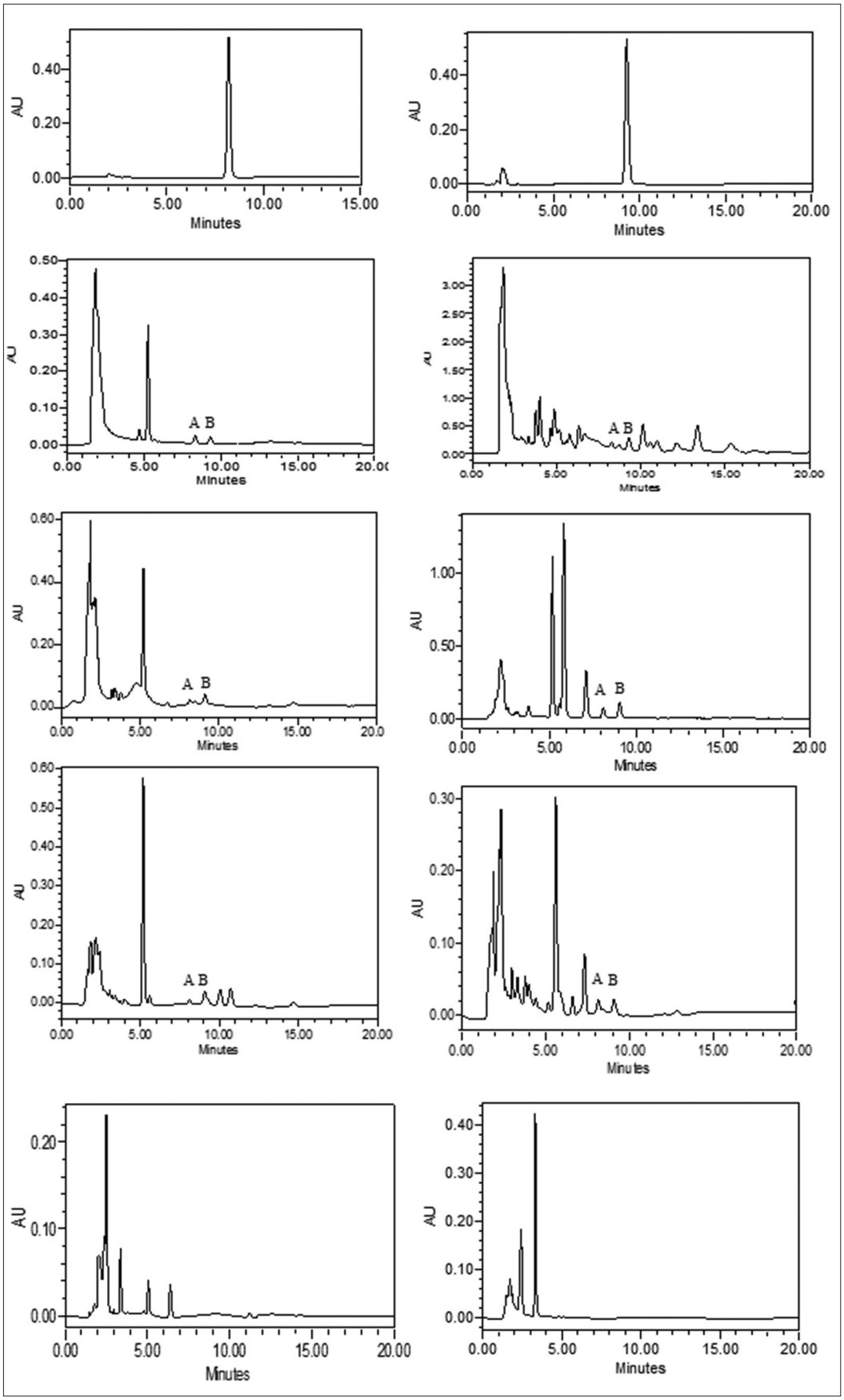

Figure 3: HPLC Chromatograms of Standards and different herbal formulations containing *Saussurea lappa*, (A).COS, (B). DE COS, (C). Saraswatha choornam, (D). Yogaraj guggulu, (e). Purim, (f). Kottamchukkadi Thailam, (G). Brahmi Grutha, (H). Coconut oil, (I). Anti-wrinkle cream, (J. Septilin Syrup

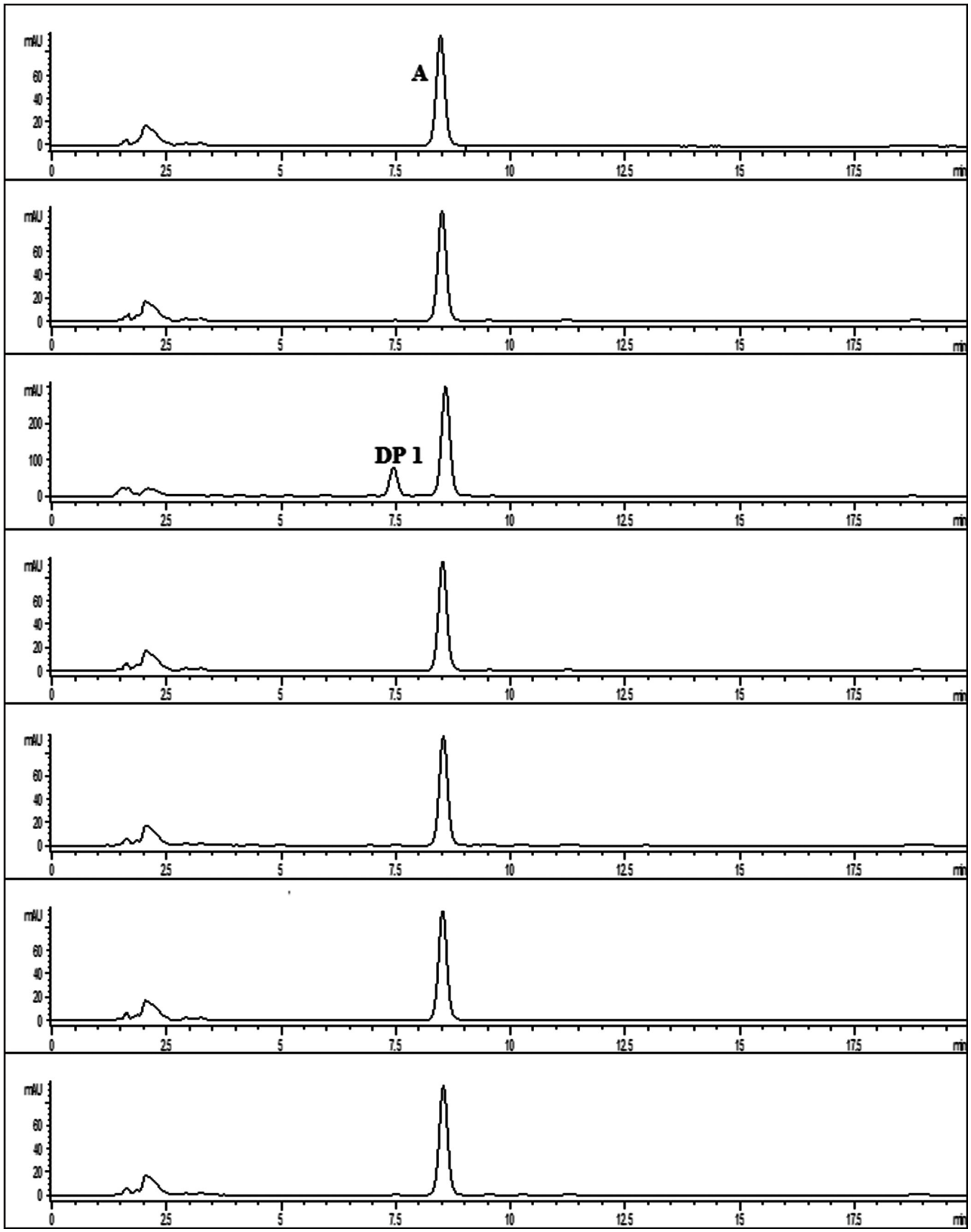

Figure 4: Chromatograms of (1) COS, (2) Acid (0.1M HCl, 120 min), (3) Alkaline (0.1M NaOH, 120 min), (4) Oxidative (6%H_2O_2, 120 min), (5) Neutral, (6) Photolytic, (7) Thermal conditions

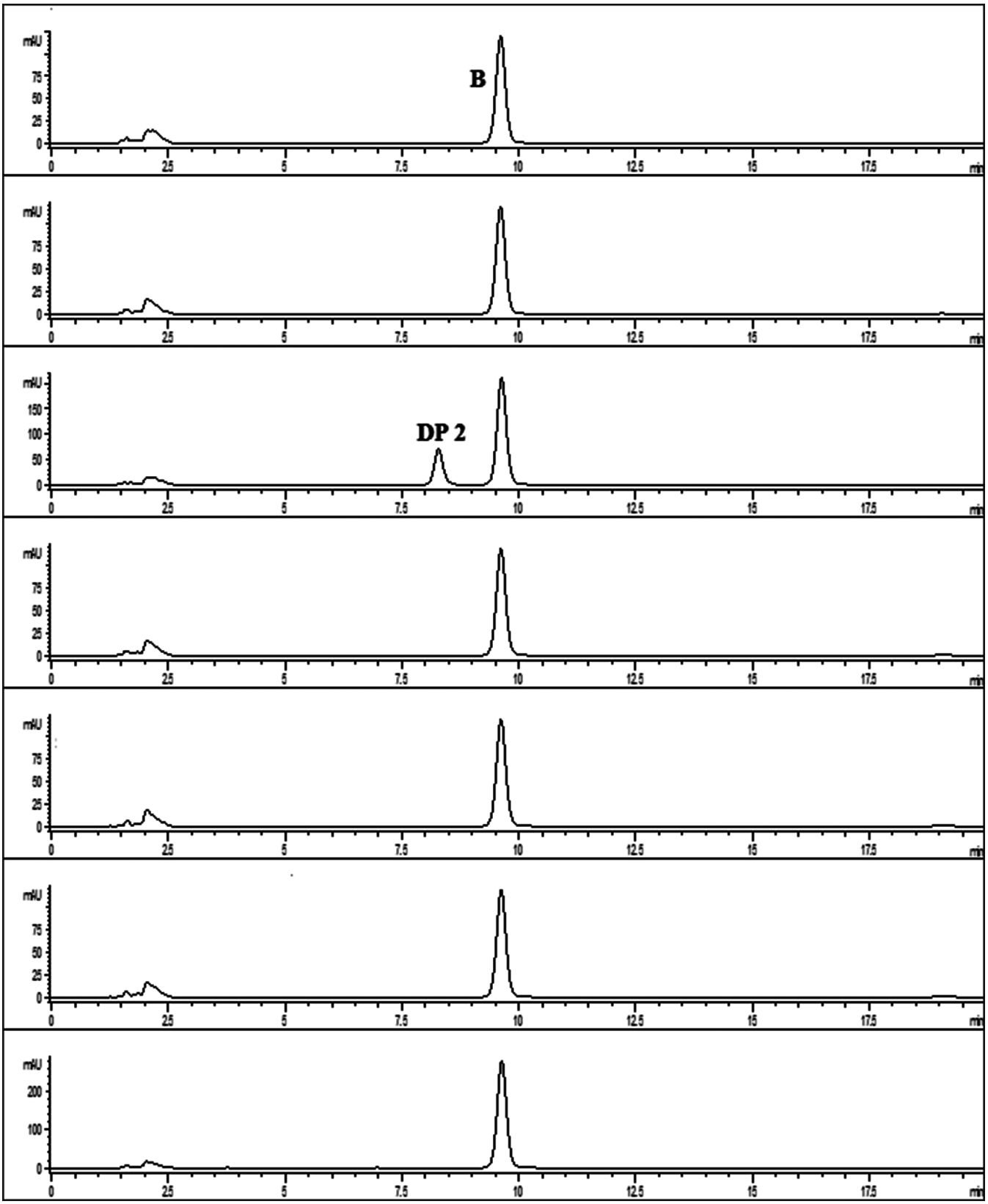

Figure 5: Chromatograms of (1) DE COS, (2) acid (0.1M HCl, 120 min), (3) Alkaline (0.1M NaOH, 120 min), (4) Oxidative (6%H_2O_2, 120 min), (5) Neutral, (6) Photolytic, (7) Thermal conditions

tablet was weighed. For oil directly 1.0 ml was taken for extraction. For all the above samples 2.5 ml of extraction solvent was added, followed by sonication for 30 min and centrifuge at 4000 rpm for 20 min. The supernatant liquid

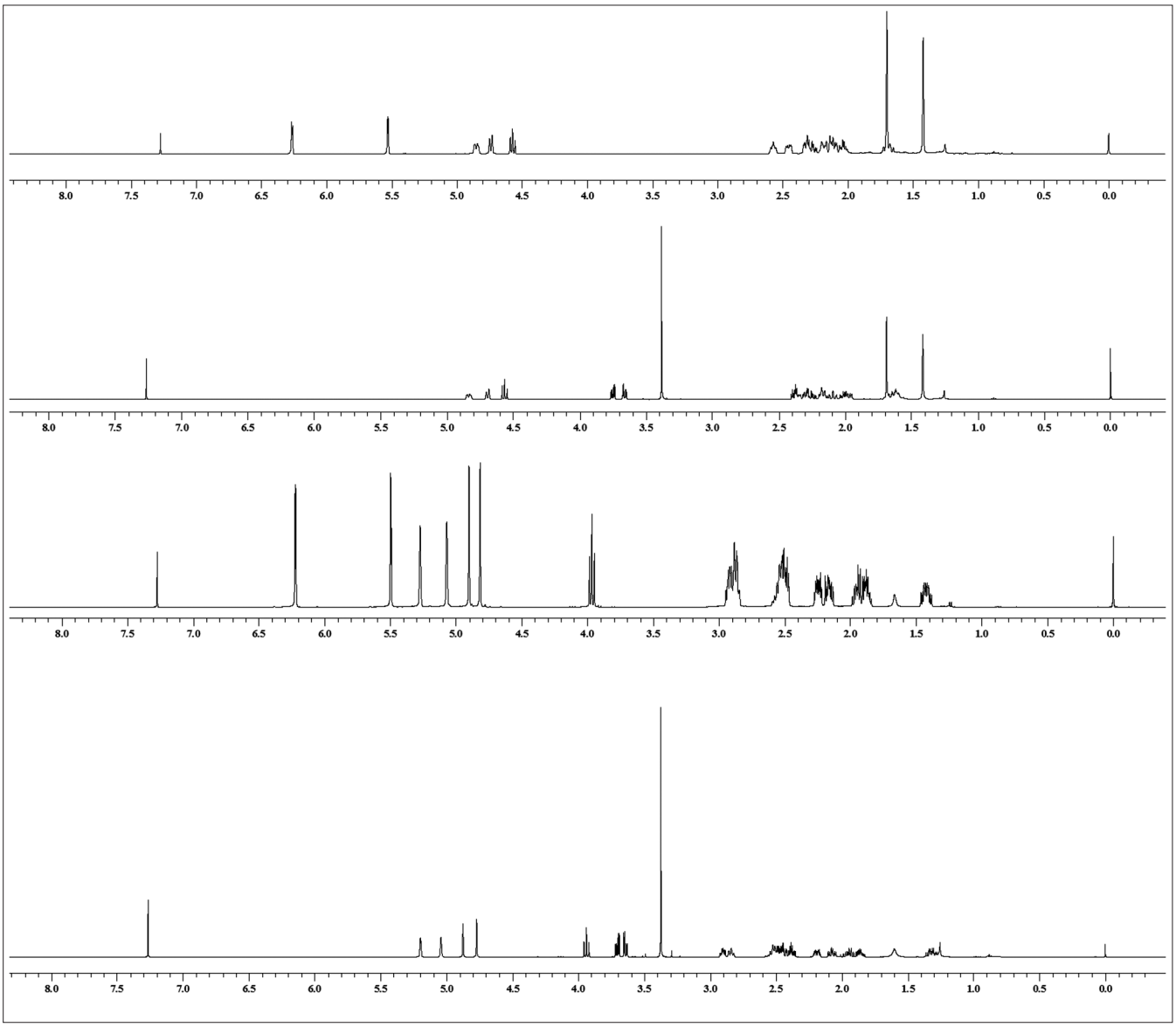

Figure 6: 1H NMR spectra of (1) COS, (2) DP-1, (3) DE COS and (4) DP-2

was decanted; the same extraction cycle was repeated three times. The resultant solution was concentrated to dryness and 100 mg/ml solution was prepared from the above samples. The prepared samples were injected directly on to HPLC system.

Preparation of standard solution

Stock solutions were prepared separately by weighed amounts (10 mg) of COS and DE COS were dissolved in methanol (10 ml) to obtain 1.0 mg/ml. Calibration standards were prepared by diluting to appropriate volume stock solution obtain the concentration ranging from 5 to 100 μg/ml for both the analytes.

Sample preparation for stability studies

The stability samples of acid and base hydrolysis were neutralized with sodium hydroxide (NaOH) and hydrochloric acid (HCl), respectively. Further dilution was carried out with deionized water to obtain 100 μg/ml other collected samples from neutral, oxidative and photolytic conditions were diluted 10 times with deionized water. All the samples were filtered through a 0.45 μm membrane filter before HPLC analysis.

Stability study

Stability studies of COS and DE COS were carried out according to ICH (2003) guidelines. About 10.0 mg of COS and DE COS was subjected to degradation under acidic, basic and neutral conditions with 5 ml methanol and 5 ml of 0.1M HCl, 0.1M NaOH, 6% H_2O_2 and water, respectively, at room temperature for 120 min. COS and DE COS were exposed to sunlight in solid as well as in solution forms for 24 h under photolytic conditions.

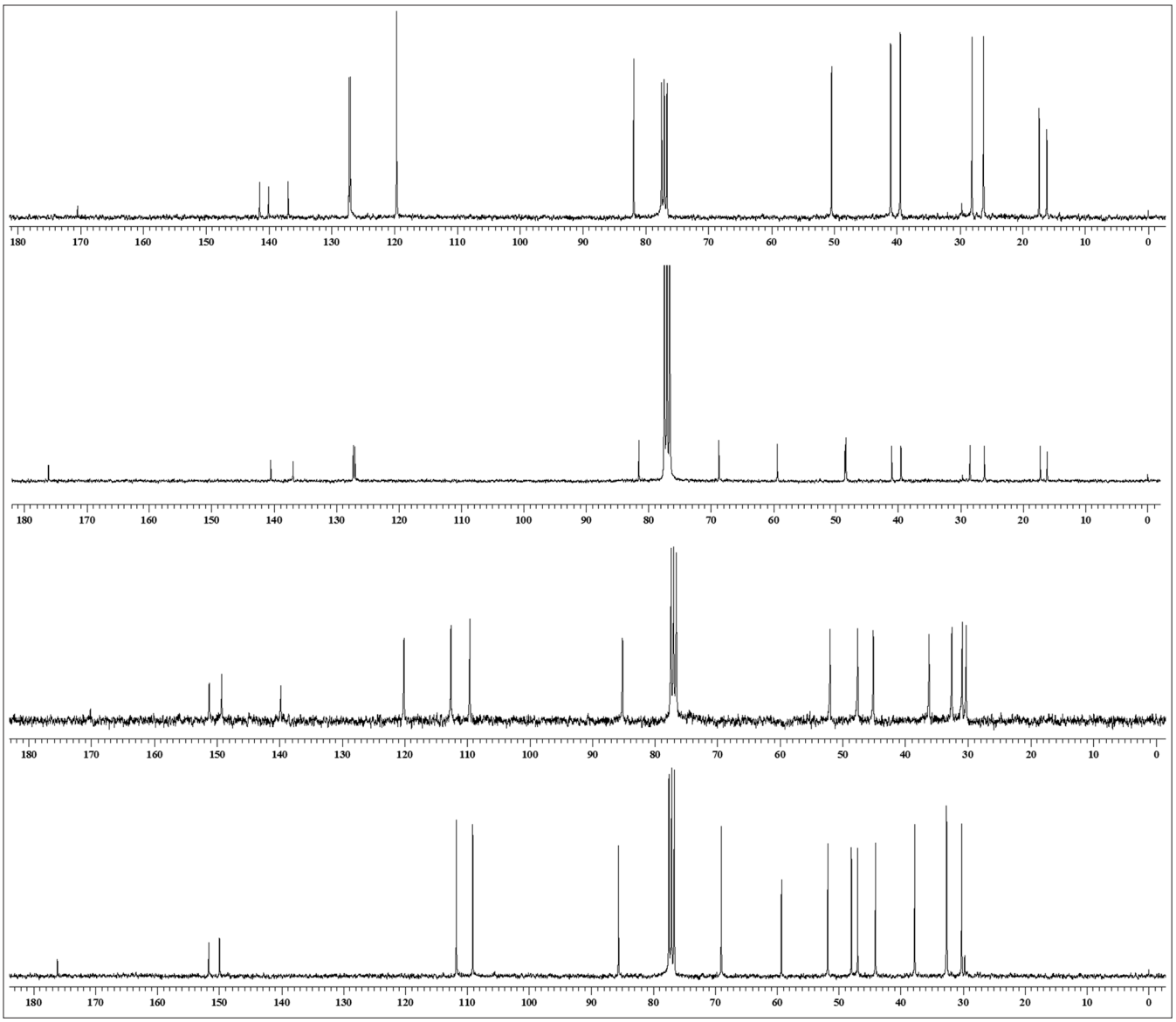

Figure 7: ^{13}C NMR spectra of (1) COS, (2) DP-1, (3) DE COS and (4) DP-2

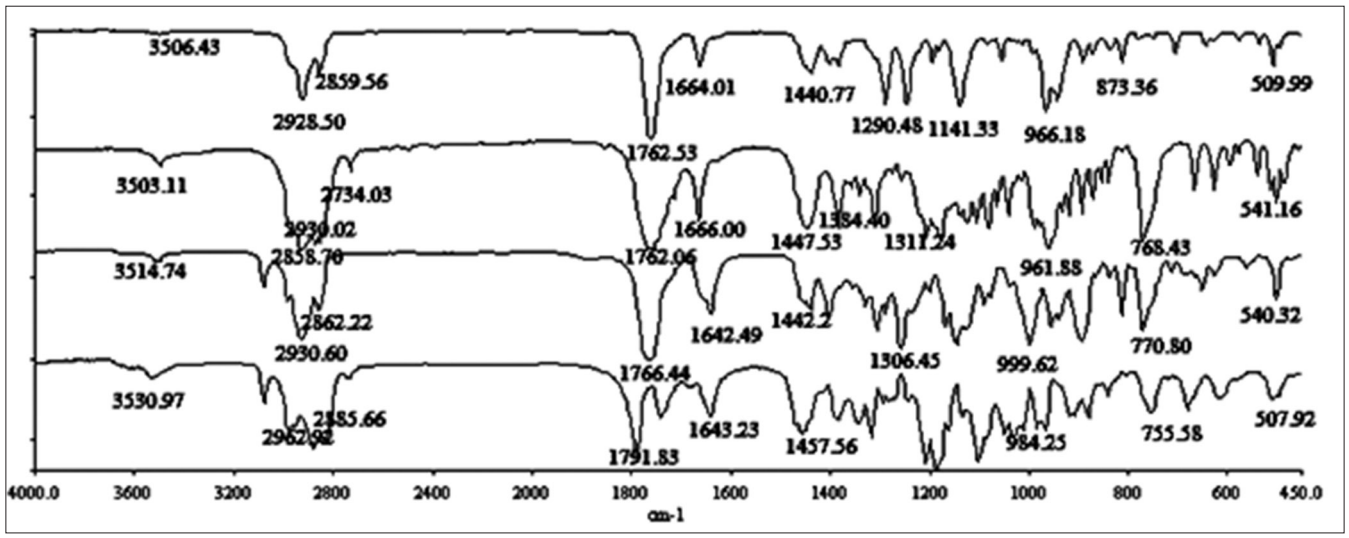

Figure 8: FT-IR spectra of (1) COS, (2) DP-1, (3) DE COS and (4) DP-2

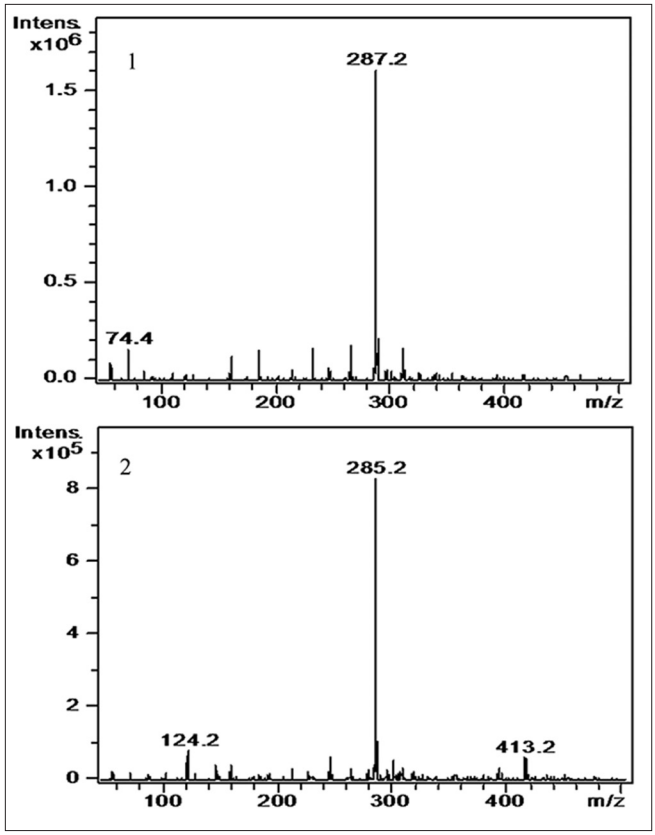

Figure 9: Positive ion mass spectra of (1) DP-1, (2) DP-2

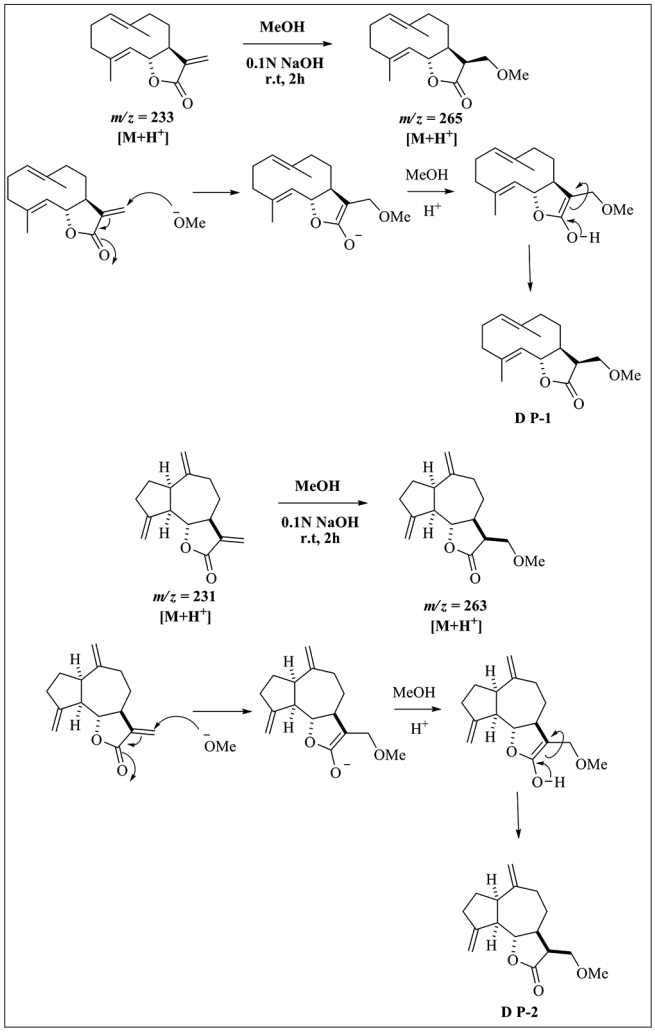

Figure 10: Degradation pathway of COS and DE COS in base hydrolysis

HPLC Analytical Conditions

HPLC analysis of *S. lappa* extract was conducted on Waters HPLC system equipped with two binary 515 pumps, 2707 auto sampler and 2998 photo diode array detection (DAD). Baseline separation was achieved using Waters NOVAPAK HR C_{18} (300 × 3.9 mm i.d., - 6 μm particle size) and was maintained at room temperature. The mobile phase used for chromatographic separation comprising of acetonitrile and water with 60:40% v/v. The flow rate was set at 1.0 ml/min and peaks were measured at 210 nm wavelength using DAD detector. For identification and confirmation of all the isolates, LC-MS analysis was performed on Agilent 1100 series LC/MSD ion trap (Agilent Technologies, Waldbronn, Germany). The flow rate was maintained at 1.0 ml/min. Detection was performed using DAD detection at λ_{max} × 210 nm. The data was processed using Waters Empower 2 system software.

Method validation

The optimized method was validated with respect to ICH Q2 (R1) (2005) guidelines. Stock solutions of the COS and DE COS were diluted to appropriate concentration for the construction of calibration curves. Five concentrations of each analyte were analyzed in triplicate and then the calibration curves were constructed by plotting the peak area versus concentration of analyte. The calibration curves were analyzed using linear regression equation and correlation coefficient.

Intra- and inter-day variations were chosen to determine the precision of the developed assay. For intra-day variability test, the mixed standards solutions were analyzed for three replicates within a day, while for inter-day variability test, the solution was examined in three replicates for consecutive three days. Variations were expressed by the percent relative standard deviation (% RSD) for intra-and inter-day. The deviation from the nominal concentration was defined as accuracy. The recovery test was performed by addition of known amount of individual standards to a predetermined sample. Three replicates were performed for the test. The limit of detection (LOD) was calculated by taking an S/N ratio of 3 as criterion, while the acceptance criterion for the limit of quantification (LOQ) was calculated by taking an S/N ratio of 10.

Table 1: Limit of detection and limit of quantification of costunolide and dehydro costus lactone

Markers	Linearity range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Correlation coefficient	Linear equation
Costunolide	5-100	1.5	4.6	>0.999	y=92180x+22981
Dehydrocostus lactone	5-100	1.3	4.0	>0.999	y=125752x+222498

LOD: Limit of detection, LOQ: Limit of quantification

Table 2: Accuracy intra and interday precision (%RSD) of methods

Compound	Amount added (µg/ml)	Amount found (µg/ml)	%RSD	Recovery (%)
Intraday; (*n*=3)				
Costunolide	5	4.98±0.01	0.20	99.60
	20	19.87±0.14	0.72	99.30
	100	99.72±0.20	0.20	99.72
Dehydrocostus lactone	5	5.03±0.03	0.59	100.60
	20	20.08±0.01	0.04	100.40
	100	100.91±0.46	0.45	100.91
Inter day (*n*=3)				
Costunolide	5	4.95±0.04	0.92	99.00
	20	20.01±0.03	0.15	100.05
	100	99.39±0.16	0.16	99.39
Dehydrocostus lactone	5	5.09±0.005	0.11	101.80
	20	20.01±0.18	0.90	100.50
	100	99.82±0.13	0.13	99.82

RSD: Relative standard deviation

Table 3: Content of costunolide and dehydrocostus lactone in different polyherbal formulations

Herbal formulations	Nature	Content of COS (mg/100g)	Content of DE COS (mg/100g)
Saraswatha choornam	Powder	0.0696	0.0054
Yogaraj guggulu	Tablet	0.0475	0.0858
Purim	Tablet	0.0516	0.0425
Kottamchukkadi Thailam	Oil	0.0481	0.0111
Brahmi Grutha	Semi solid	0.0012	0.0065
Lakshadi Coconut oil	Oil	0.0055	0.0566
Septilin Syrup	Liquid	Not detected	Not detected
Anti-wrinkle cream	Cream	Not detected	Not detected

DE COS: Dehydrocostus lactone costunolide, COS: Costunolide

RESULTS AND DISCUSSIONS

Optimization of extraction solvent

Fine powdered roots of *S. lappa* approximately 500 mg were extracted using different solvents, namely hexane, ethyl acetate, chloroform and methanol. An aliquot of 2.5 ml of extraction solvent was added to 500 mg *S. lappa* roots in 15 ml centrifuge tube, sonicated for 30 min followed by centrifuge for 20 min at 2500 rpm. The supernatant liquid was collected separately in 10 ml volumetric flask. 2.5 ml of solvent was added to the residue and same cycle was repeated for three more times. The volume was made to the mark in volumetric flask with extraction solvent. The same procedure was followed for all the solvents used in this study. The solution was filtered through 0.45 µm syringe filter. The filtered solution was used for HPLC analysis. The peak areas of the standard compounds were compared among the extraction solvents tested for this study.

The extraction efficacy of different solvents on COS and DE COS extraction from *S. lappa* roots is evaluated and shown in Figure 2. Among the different solvents screened for the extraction, methanol was found to have the highest extractability.

Method validation

The HPLC method for the quantitative analysis of COS and DE COS was validated in terms of its linearity, LOD, LOQ, precision and recovery. For linearity, the peak area responses for various concentrations of COS and DE COS (5, 10, 20, 50 and 100 µg/ml) were obtained under the optimized HPLC conditions. The analyte concentration and peak area were plotted. The calibration curve thus constructed was found to be linear in a concentration range of 5-100 µg/ml with a regression coefficient values (r) of COS and DE COS being 0.9999 and 0.999, respectively. The regression equation for the plot was $y = 92180x–22981$, $y = 12575x–22249$, where y is the peak area at 210 nm and x is the COS and DE COS concentration in µg/ml. LOD and LOQ were estimated from the signal-to-noise ratio and were found to be 1.5 and 4.6 µg/ml respectively for COS and for DE COS were found to be 1.3 and 4.0 µg/ml, respectively [Table 1].

The intra- and inter-day variations (%RSD) of COS (0.15-0.92%) and DE COS (0.04-0.90%) were within acceptable ranges. Recovery was determined to evaluate the accuracy of the method. The data on recovery were obtained by known concentration of standard COS and DE COS in the low, medium and higher ranges (5, 20 and 100 µg/ml) [Table 2].

The recovery values were within acceptable limits of COS (99.3-100.05%) and DE COS (99.82-101.80%).

Estimation of COS and DE COS in polyherbal formulations

The applicability of the method for the quantification of COS and DE COS in phytopharmaceuticals was

Table 4: Spectral data of degradation products

Compound	^{1}H NMR 400 M Hz, $CDCl_3$	^{13}C NMR 100 M Hz, $CDCl_3$	FT-IR (cm^{-1})	Observed mass (m/z) by LC-ESI/MS	HRMS calculated	HRMS found
DP-1	δ 4.83 (1H, dd, *J* = 3.6, 11.2 Hz), 4.69 (1H, d, *J*= 9.7 Hz), 4.56 (1H, t, *J* = 9.6 Hz), 3.75 (1H, dd, *J* = 10.0, 3.9 Hz), 3.66 (1H, dd, *J* = 10.0, 3.9 Hz), 3.38 (3H, s), 2.41-2.34 (2H, m), 2.32-2.22 (2H, m), 2.20-2.11 (2H, m), 2.09-2.06 (1H, m), 2.04-1.94 (2H, m), 1.69 (3H, s), 1.65-1.59 (1H, m), 1.41 (3H, s):	δ 176.0, 140.4, 136.9, 127.2, 126.9, 81.5, 68.6, 59.3, 48.4, 48.3, 40.9, 39.5, 28.4, 26.1, 17.2, 16.1.	aromatic C-H stretch 2858.70 cm^{-1}; C=O stretch 1762.06 cm^{-1}; C=C stretch 1666.00 cm^{-1}; C-O stretch 1447.53 cm^{-1};	[M+Na$^+$]=264	264.1618	264.1615
DP-2	δ 5.2 (1H, d, *J* = 2.0 Hz), 5.0 (1H, d, *J* = 2.0 Hz), 4.87 (1H, s), 4.76 (1H, s), 3.93 (1H, t, *J* = 9.3 Hz), 3.70 (1H, dd, *J* = 4.2, 9.7 Hz), 3.63 (1H, dd, *J* = 4.2, 9.7 Hz), 3.37 (3H, s) ,2.93-2.87(1H, m), 2.83(1H, t, *J* = 9.7 Hz), 2.57-2.42(4H, m), 2.40-2.34(1H, m), 2.21-2.15 (1H, m), 2.10-2.03 (1H, m), 1.98-1.90 (1H, m), 1.88-1.81 (1H, m), 1.36-1.27 (1H, m).	δ 176.1, 151.1, 149.9, 111.7, 109.9, 85.5, 68.9, 59.2, 51.7, 47.9, 46.9, 44.0, 37.7, 32.6, 32.5, 30.1,	aromatic C-H stretch 2858.70 cm^{-1}; C=O stretch 1762.06 cm^{-1}; C=C stretch 1666.00 cm^{-1}; C-O stretch 1447.53 cm^{-1};	[M+Na$^+$]=262	262.1642	262.1650

demonstrated with eight polyherbal formulations belonging to three different classes, namely choornam, thailam and tablets. The selected formulations had *S. lappa* as an important ingredient and considerable differences in their composition and method of preparation. The chromatographic separation of COS and DE COS present in commercial herbal formulations is shown in Figure 3. The content of COS and DE COS in polyherbal formulations is shown in Table 3. It is important to mention that COS and DE COS were not detected in formulations of septilin syrup and anti-wrinkle cream [Figure 3I and J].

Stability studies

The stability studies of COS and DE COS under various conditions was investigated by HPLC. The obtained chromatograms confirm that COS and DE COS is susceptible to degradation under alkaline hydrolysis. Although the markers were subjected to neutral (water), acid (0.1 M HCl) and base (0.1 M NaOH) hydrolysis, oxidative (6% H_2O_2) and photolytic conditions, it remained stable in all the conditions except for base hydrolysis.

COS and DE COS in methanol with 1M NaOH at room temperature (1 mg/ml) for 120 min, two degradation products (DP-1 and DP-2) were obtained. The two base hydrolysis products were obtained as shown in Figures 4 and 5. The degradants were characterized by LC-ESI-MS, ^{1}H, ^{13}C-NMR and FT-IR spectroscopy as shown in Figures 6-9. Spectral data was shown in Table 4. The degradation pathways are show in Figure 10.

From above-mentioned characterization studies of resulting compounds in alkali hydrolysis it is assumed that formation of compounds (DP-1, DP-2) is due to the formation of sodium methoxide in the presence of methanol as a solvent.

CONCLUSION

The method was validated for linearity, precision, accuracy, LOD and LOQ. The method is simple, accurate and precise and may be recommended for routine quality control analysis of *S. lappa* root extracts and its polyherbal formulations. Stability studies of markers were developed. The behavior of markers under various stability conditions was studied. The degradation products were characterized and the possible pathways of degradation were proposed.

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REFERENCES

1. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003;3:768-80.
2. Gupta V, Bansal P, Kumar S, Sannd R, Rao MM. Therapeutic efficacy of Phytochemicals as anti-anxiety-a review. J Pharm Res 2010;3:174-9.
3. Adams RP. Yields and seasonal variation of phytochemicals from Juniperus species of the United States. Biomass 1987;12:129-39.
4. Burda S, Oleszek W, Lee CY. Phenolic compounds and their changes in apples during maturation and cold storage. J Agric Food Chem 1990;38:945-8.
5. Kalt W, Forney CF, Martin A, Prior RL. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. J Agric Food Chem 1999;47:4638-44.

6. Okarter N, Liu, CS, Sorrells ME, Liu RH. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. Food Chem 2010;119:249–57.
7. Paul A, Bawdekar AS, Joshi RS, Somashekara Rao K, Kelker GR, Battacharya SC. Terpenoids XX: examination of costus root oil. Perf Ess Oil Rec 1960;15:115-20.
8. Rao AS, Kelkar GR, Bhattacharya SC. Terpenoids-xxv* Structure of Saussurea lactone. Tetrahedron 1961;13:319-23.
9. Dhillon RS, Kalsi PS, Singh WP, Gautam VK, Chhabra BR. Guaianolides from *Saussurea lappa*. Phytochemistry 1987;26:1209-10.
10. Mathur SB, Hiremath SV, Kulakarni GH, Kelkar GR, Bhattacharyya SC, Simonovic D, *et al*. Terpenoids-LXX: Structure of dehydro costuslactone. Tetrahedron 1965;21:3575-90.
11. Rao AS, Kelkar GR, Bhattacharya SC. The structure of costunolide, a new sesquiterpene lactone from costus root oil. Tetrahedron 1960;9:275-83.
12. Luna HJ, Costa MC, Gonzalez HG, Rodrigues AI, Castilho PC. Synergistic antimycobacterial activities of sesquiterpene lactones From *Laurus spp*. J Antimicrob Chemother 2007;59:548-52.
13. Wedge DE, Galindo JC, Macias FA. Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. Phytochemistry 2000;53:747-57.
14. Fischer NH, Lu T, Cantrell CL, Castañeda-Acosta J, Quijano L, Franzblau SG. Antimycobacterial evaluation of germacranolides. Phytochemistry 1998;49:559-64.
15. Ohnishi M, Yoshimi N, Kawamori T, Ino N, Hirose Y, Tanaka T, *et al*. Inhibitory effects of dietary protocatechuic acid and costunolide on 7,12-dimethylbenz[a] anthracene-induced hamster cheek pouch carcinogenesis. Jpn J Cancer Res 1997;88:111-9.
16. Kawamori T, Tanaka T, Hara A, Yamahara J, Mori H. Modifying effects of naturally occurring products on the development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. Cancer Res 1995;55:1277-82.
17. Gu JQ, Gills JJ, Park EJ, Mata-Greenwood E, Hawthorne ME, Axelrod F, *et al*. Sesquiter-penoids from *Tithonia diversifolia* with potential cancer chemopre-ventive activity. J Nat Prod 2002;65:532-6.
18. Yamahara J, Kobayashi M, Miki K, Kozuka M, Sawada T, Fujimura H. Cholagogic and antiulcer effect of *saussureae radix* and its active components. Chem Pharm Bull 1985;33:1285-8.
19. Yoshikawa M, Hatakeyama S, Inoue Y, Yamahara J. Saussureamines A, B, C, D, and E new anti-ulcer principles from Chinese *Saussureae Radix*. Chem Pharm Bull 1993;41:214-6.
20. Koch E, Klaas CA, Rüngeler P, Castro V, Mora G, Vichnewski W, *et al*. Inhibition of inflammatory cytokine production and lymphocyteproliferation by structurally different sesquiterpene lactones corre-lates with their effect on activation of NF-kappaB. Biochem Pharmacol 2001;62:795-801.
21. Choi JY, Na MK, Hwang IH, Lee SH, Bae EY, Kim BY, *et al*. Isolation of betulinic acid, its methyl ester and guaiane sesquiterpenoids with protein tyrosine phosphatase 1B inhibitory activity from the roots of *Saussurea lappa* C.B.Clarke. Molecules 2009;14:266-72.
22. Shu D, Yue-tao L, Jing-bo P, Hong-mei J, Zhong-mei Z, Chang-yuan Y. Simultaneous HPLC determination of costunolide and dehydrocostuslactone in Xin-Ke-shu preparations. Pharm Crops 2011;2:74-8.
23. Vijayakannan R, Karan M, Dutt S, Jain V, Vasisht K. A rapid densitometric TLC method for simultaneous analysis of costunolide and dehydro-costus lactone in *Saussurea costus*. Chromatographia 2006;63:277-81.
24. Fangdi H, Shilan F, Yuquiong W, Yingyani B, Chunming W. Quantitative analysis of costunolide and dehydro costuslactone in rat plasma by ultraperformance liquid chromatography-elctrospray ionization-mass spectrometry. Biomed Chromatogr 2011;25:547-54.
25. Fangdi H, Shilan F, Yuqiong W, Yingyani B, Fang C, Yongjie L, *et al*. Development of high performance liquid chromatography method for costunolide and dehydrocostuslactone in mice plasma and tissues: Application to pharmacokinetic Study. Chin J Chem 2010;28:2293-300.
26. Liu Y, Wu H, Zhu S, Kang C, Xu H, Su Z, *et al*. Rapid determination of costunolide and dehydrocostuslactone in human plasma sample and Chinese patent medicine Xiang Sha Yang Wei capsule using HPLC-DAD coupled with second-order calibration. Chin J Chem 2012;30:1-7.
27. Robinson A, Kumar TV, Sreedhar E, Naidu VG, Krishna SR, Babu KS, *et al*. A new sesquiterpene lactone from the roots of *Saussurea lappa*: structure-anticancer activity study. Bioorg Med Chem Lett 2008;8:4015-17.
28. Rao RN, Raju SS, Babu KS, Rao VP. HPLC determination of costunolide as a marker of *Saussurea lappa* and its herbal formulations. Int J Res Pharm Chem 2013;3:99-107.

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