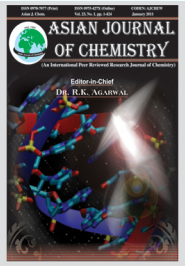
Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 631-635

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Spectrophotometric Determination of Cinnamaldehyde from Crude Drugs and Herbal Preparations

F.M.A. RIND[1,2,*], A.H. MEMON[1], F. ALMANI[1], M.G.H. LAGHARI[1], U.R. MUGHAL[1], M.L. MAHESHWARI[1] and M.Y. KHUHAWAR[2]

[1]Faculty of Pharmacy, University of Sindh, Jamshoro, Pakistan
[2]Dr. M.A. Kazi Institute of Chemistry, Faculty of Natural Sciences, University of Sindh, Jamshoro, Pakistan

*Corresponding author: Tel: +92 22 2771150; E-mail: mahboobalirind@yahoo.com

(*Received*: 24 February 2010; *Accepted*: 24 September 2010) AJC-9126

A new spectrophotometric method has been examined for the analysis of cinnamaldehyde from cinnamon bark, cinnamon oils and herbal preparations. The method is based on the reaction of cinnamaldehyde through its aldehyde group with α-aminonaphthalene. The derivative formed exhibit a maximum absorbance which was measured at 376 nm with molar absorbtivity of 5.8×10^3 L mol/cm. A linear relationship was obtained between the absorbance and the concentration of the derivative which obeyed the Beer's law within 05-25 µg/mL. Cinnamaldehyde was extracted from cinnamon in ethanol. At the optimized conditions, the method was applied for the determination of cinnamaldehyde and amounts found were from cinnamon bark, cinnamon oils and herbal preparations 0.40-0.57, 0.44-46.00 and 0.003-0.340 % with (RSD) 0.01-0.03 %, 0.02-1.52 % and (RSD) 0.015-1.99 % (n=3), respectively.

Key Words: Cinnamaldehyde, α-Aminonaphthalene, Spectrophotometry.

INTRODUCTION

Cinnamaldehyde (CA) is the main component of cassia oil as well as cinnamon bark oil and is used in flavoring compounds to impact cinnamon flavor. It is a potent antimicrobial agent, antidiarrhoeal, antipyretic, sedative and possesses insecticidal properties[1]. Several methods have been reported for the determination of cinnamaldehyde based on titrimetry[2], second derivative ultra violet spectrometry[3], fluorimetry[4,5], thin layer-chromatography[6,7], liquid chromatography[8-15] and gas chromatography[16-21]. The spectrophotometric determination is carried out either using the natural absorbance of cinnamaldehyde at 286 nm or is derivatized with a suitable derivatizing reagent. Derivatization either increases the molar absorptivity or shifts the λ_{max} of the drug towards visible[22]. In the present work α-aminonaphthalene (AN) is used as a derivatizing reagent for selective determination of cinnamaldehyde spectrophotometrically.

EXPERIMENTAL

All the chemicals and reagents used were of analytical or pharmaceutical grades. The double distilled water used throughout the study was obtained from distillation plant all made of glass. Pure cinnamaldehyde (CA), α-aminonaphthalene (AN) and acetic acid were obtained from (E. Merck, Germany). Sodium acetate (Fluka, Switzerland) and ethanol from (BDH, UK) were used.

Buffer solutions between pH 1-10 at unit interval were prepared from hydrochloric acid (1 M), potassium chloride (1 M), acetic acid (1 M), sodium acetate (1 M), sodium bicarbonate (1 M), sodium carbonate (saturated solution), ammonium chloride (1 M) and ammonia solution (1 M).

The solution of AN 1 % (w/v) was prepared in ethanol. The spectrophotometric studies were carried out with a double beam spectrophotometer UV-1601, Shimadzu Corporation Japan.

Preparation of cinnamylidene-α-aminonaphthalene (CA-AN): Cinnamaldehyde (1 mL) in ethanol (5 mL) was added to a solution of α-aminonaphthalene (1 g) in ethanol (5 mL) and was added hydrochloric acid (0.5 mL 6 M). The contents were refluxed for 45 min and cooled overnight. The precipitate obtained was filtered and recrystalized from ethanol, m.p. 168 ºC calcd. (%) for $C_{19}H_{15}N$, expected C = 88.7, H = 5.8, N = 5.5, found (%) C = 87.8, H = 5.5, N = 4.5, IR (KBr, ν_{max}, cm^{-1}) indicates bands at 3055, 2969 (CH), 1621(C=N), 1575, 1558, 1519, 1538, (C=C), 746 and 691 (CH) vibrations (Fig. 1).

The absorbance in ethanol at λ_{max} 376 nm against reagent blank was recorded with molar absorptivity 5.8×10^3 L mol^{-1} cm^{-1}.

Analytical procedure: The ethanolic solution (0.5-2.5 mL) containing CA (50-250 µg) was transferred to 10 mL stoppered volumetric flasks and was added 3 mL AN 1 % in

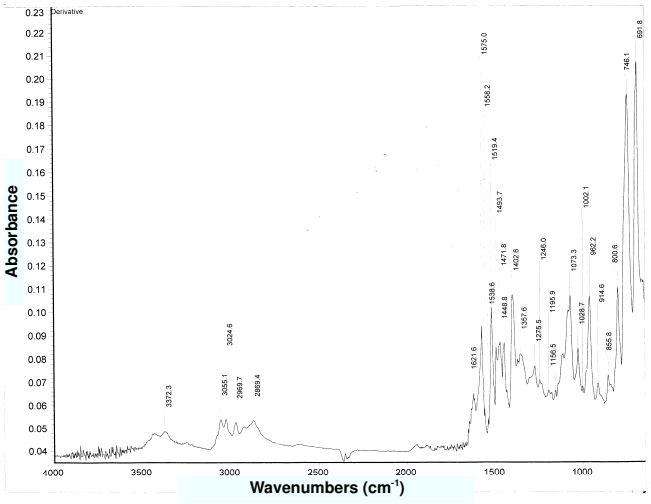


Fig. 1. IR spectrum of pure CA-AN derivative

ethanol (w/v), followed by acetate buffer pH 4 (1 mL). The contents were heated on water bath at 90 ºC for 20 min. The solution was cooled at room temperature and the volume was adjusted to mark with ethanol. The absorbance was measured at 376 nm against reagent blank, prepared in a similar way, omitting the addition of cinnamaldehyde.

Analysis of cinnamaldehyde from cinnamon barks: Two brands of cinnamon bark, one as unbranded and another as Srilankan cinnamon were obtained from market. The cinnamon bark, 1 g from each brand was taken in separate stainless steel pestles and mortars and was ground to the powder. Each powdered sample was soxhleted with ethanol (100 mL) for 24 h. The extracts were filtered twice using whatmann filter paper No. 42 and the volume was adjusted to 100 mL with ethanol. Finally the sample (1 mL) from each was taken in separate volumetric flasks (10 mL) and the procedure was repeated as described in analytical procedure. The amounts of cinnamaldehyde in both the cinnamon samples were computed using the external calibration curve prepared from standard solutions of cinnamaldehyde. The results were also compared with those obtained from the calibration curve based on natural absorbance of cinnamaldehyde at 286 nm.

Reference UV (un-derivatized) method: The solution (0.5-2.5 mL) containing CA (5-25 µg) was transferred to 10 mL stoppered volumetric flasks and volume was adjusted to mark with ethanol. The absorbance was measured at 286 nm against ethanol with molar absorptivity 3.7×10^4 L mol^{-1} cm^{-1}. The amount of the drug was computed from its calibration curve.

Analysis of CA from cinnamon oils: For the analysis of CA from cinnamon oils, three brands of cinnamon oils namely Naedern Holland b v, Haque planters international (HQ oil) Karachi and standard flavour company Lahore (SFC oil) as Unani remedy available in the local market were analyzed.

The sample 0.05 mL from Naedern oil, 0.5 mL from HQ oil and 1 mL from SFC oil were diluted to 50 mL. The sample (0.5 mL) from each diluted solution was taken in three separate volumetric flasks (10 mL) and the procedure was repeated as described in analytical procedure.

Analysis of cinnamaldehyde from herbal preparations: For the analysis of CA contents from herbal preparations, ten brands of herbal syrups namely Carmizol (Ihsan Herbal Pharmacy Lahore, Pakistan), Gastomy (Riaz Dawakhana Faisalabad, Pakistan), Gastokil (Bara Dawakhana Faisalabad, Pakistan), Kisirin (Tayyabi Dawakhana (Pvt.) Ltd. Karachi, Pakistan), Gasnil (Marhaba Laboratories Kot lakhpat Lahore, Pakistan), Gasto (Ahsan Laboratories Karachi, Pakistan), Gastofill (Qarshi Industries (Pvt.) Ltd. Quetta, Pakistan), Gastol (Ashraf Laboratories (Pvt.) Ltd. Faisalabad, Pakistan), Gastovena (Lasani Pharma (Pvt.) Ltd. Lahore, Pakistan), Gaskil (Top Treatments Herbal Division Multan Road Lahore, Pakistan), containing extract of cinnamon bark were analyzed.

The syrup sample (10 mL) from each of the Carmizol, Gastomy, Gastokil, Kisirin, Gasto, Gasnil and 25.00, 16.60, 0.50 and 0.84 mL from each of Gastofill, Gastol, Gastovena and Gaskil, respectively were diluted to 50 mL with ethyl alcohol in separate volumetric flasks and filtered. Finally 3 mL from each was taken in separate 10 mL volumetric flasks and the procedure was repeated as described in the above analytical procedure. The quantitation was carried out using external calibration curve.

RESULTS AND DISCUSSION

Cinnamaldehyde (CA) reacts with α-aminonaphthalene (AN) to form a azomethine derivative, cinnamylidene-α-aminonaphthalene (CA-AN) (Fig. 2), which absorbs maximally with bathochromic shift at 376 nm against reagent blank with molar absorptivity of 5.8×10^3 L mol/cm. Therefore AN was examined as a derivatizing reagent for the spectrophotometric determination of CA. The different parameters which effect the formation of derivative and accuracy of the analysis, such as analytical wavelength, reagent concentration, order of mixing the reagents, heating time and temperature, the solvent, pH, stability of derivative and interference of associated matter were optimized carefully.

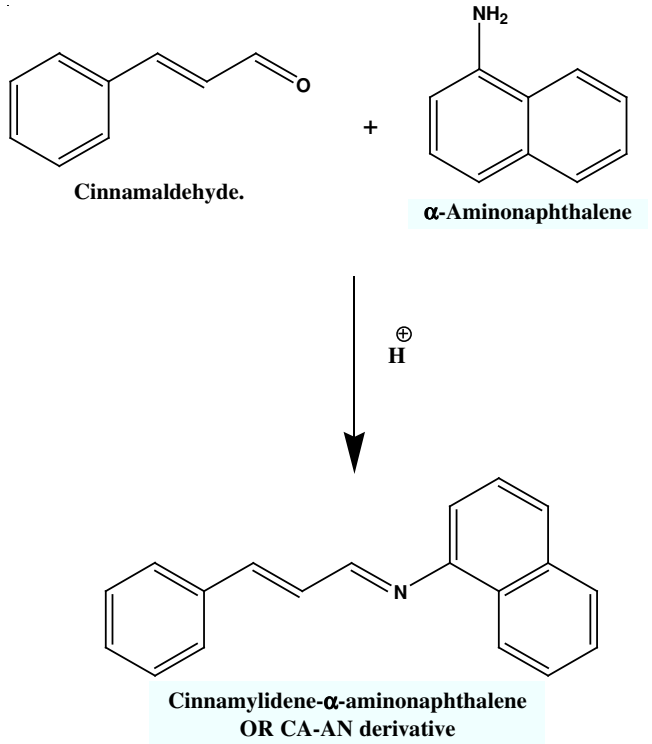


Fig. 2. Formation of derivative CA-AN by reaction of CA with AN

Optimization of parameters

Analytical wavelength: For the quantitative analysis, the wavelength of maximum absorbance plays important role because some times derivatizing reagent may absorb close to region where the analyte derivative absorb. This may cause error in absorption of the drug because the derivatizing reagent is added in excess to complete the reaction quantitatively. To avoid this problem it is necessary to select the wavelength where the derivatizing reagent indicates minimum absorbance and maximum absorbance value for analyte. The absorbance value of 5 µg/mL of CA as AN derivative was recorded at different wavelengths after heating for 20 min at 90 ºC and pH 4. It is evident that the maximum absorbance occurs at 376 nm. It was therefore, the wavelength of 376 nm selected as λ_{max}.

Effect of reagent concentration: The effects of adding various amounts of AN solution on absorbance of 5 µg/mL CA is given in Fig. 3. The concentration of reagent AN was varied between 1-5 mL of 1 % in ethanol with an interval of 1 mL and absorbance was measured at λ_{max} 376 nm. It is observed that the maximum absorbance occurred in the presence of 3 mL of the reagent AN.

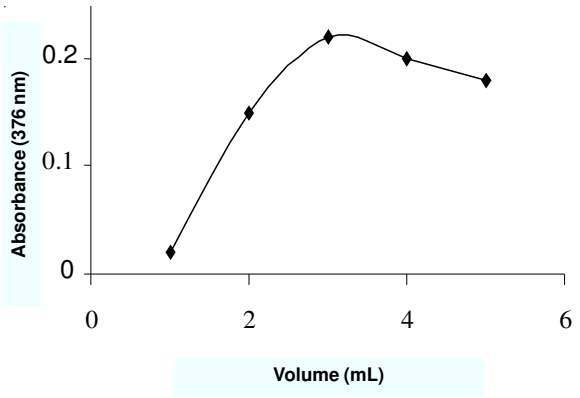


Fig. 3. Effect of reagent concentration on absorbance of cinnamaldehyde derivative

Effect of order of mixing the reagents: The order of adding reagents during derivatization process has important role in accuracy of results and enhancement of absorbance.

In the present study it was observed that the addition of buffer pH 4 (1 mL) to 5 µg/mL cinnamaldehyde solution (0.5 mL) followed by the reagent (3 mL) resulted in a lower absorbance value. Taking the reagent first and adding the buffer followed by cinnamaldehyde solution also gave lower absorbance value. The maximum absorbance value was observed when, the solution of CA standard was added 3 mL of AN 1 % in ethanol (w/v) followed by acetate buffer pH 4 (1 mL) in a volumetric flask (10 mL). The contents were heated on water bath with thorough mixing and the volume was adjusted to the mark with ethanol.

Optimization of heating time and temperature for the formation of derivative: To get maximum absorbance value for an analyte, the selection of proper time and temperature for the formation of stable derivative are essential parameters. The effect of heating time and temperature on absorbance of 5 µg/mL CA solution in the presence of 1 % AN solution was checked at 376 nm from 0-0.5 h at 90 ºC with an interval of 5 min. The results are presented in (Fig. 4). It is evident from figure that a similar absorbance was observed after heating for 15 min and the heating time of 20 min at 90 ºC was considered as optimal.

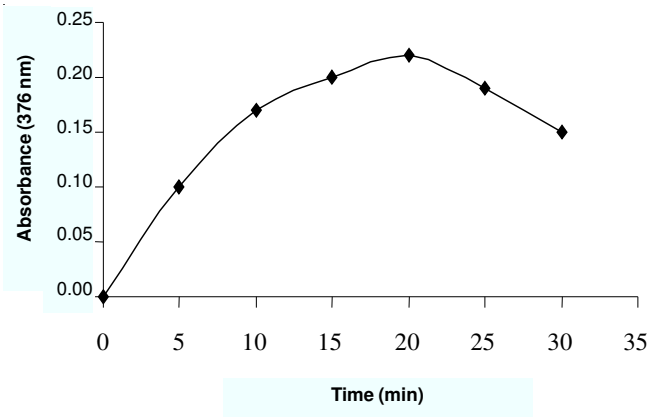


Fig. 4. Effect of heating time on derivatization of cinnamaldehyde

Effects of solvents: The effect of various solvents such as methanol, 1-propanol, 2-propanol, 1-butanol, amyl alcohol, 1,2-propandiol, acetonitrile, ethyl acetate and tetrahydrofuran was investigated on the absorbance of 10 µg/mL CA. Each of the solvent 1and 2 mL were added after the addition of 1 % ethanolic solution of AN and 1 mL acetate buffer pH 4 followed by heating for 20 min. The results are summarized in (Table-1). The ethanol showed to be the best choice while the addition of H_2O produced turbidity.

TABLE-1
EFFECTS OF MIXING OF VARIOUS ORGANIC POLAR SOLVENTS TO 10 µg/mL CA-AN DERIVATIVE

Solvents	Volume (mL) added	Absorbance (376 nm)	Effect (%)
Ethanol	–	0.448	0.00
Methanol	1	0.448	0.00
	2	0.449	+0.22
1-Propanol	1	0.442	-1.33
	2	0.443	-1.11
2-Propanol	1	0.439	-2.00
	2	0.442	-1.11
1-Butanol	1	0.404	-9.82
	2	0.421	-6.02
Amyl alcohol	1	0.404	-9.82
	2	0.431	-3.79
1,2-Propandiol	1	0.462	+3.12
	2	0.463	+3.34
Acetonitrile	1	0.467	+4.24
	2	0.470	+4.91
Ethyl acetate	1	0.398	-11.16
	2	0.440	-1.78
Tetrahydrofuran	1	0.129	-71.20
	2	0.188	-61.47

Effect of pH: The effect of adding 1 mL of 1 M buffers of pH range 1-10 on the absorbance of 5 µg/mL CA solution per 10 mL at already optimized conditions were studied and the results are shown in Fig. 5.

It is evident from Fig. 5 that in highly acidic medium *i.e.*, at pH 1 and 2, the absorbance is low, while at the pH 8-10

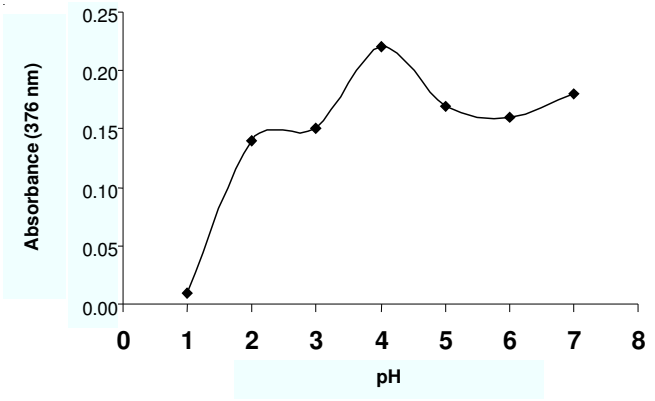


Fig. 5. Effect of pH on derivatization of cinnamaldehyde

precipitation occurred. The absorbance increased with acidic buffer at pH 3 and it becomes maximum at pH 4 and then it decreased upto pH 7. Therefore the acetate buffer of pH 4 was selected as optimal.

Interference study: The effect of possible presence of associated materials such as mannitol, sorbitol, lactose, sucrose, glucose, galactose and fructose was investigated at 10 times the concentration of CA and it was observed that none of these substances interfered and change in absorbance as compared to standard was less than 5 % (Table-2).

TABLE-2
EFFECTS OF DIFFERENT POSSIBLE ADDITIVES ON THE ABSORBANCE OF 10 µg/mL CA-AN DERIVATIVE

Chemical added	Absorbance (376 nm)
–	0.448
Mannitol	0.447
Sorbitol	0.448
Glucose	0.449
Galactose	0.445
Fructose	0.448
Sucrose	0.449

Stability of the derivative: The stability of CA-AN derivative was examined in terms of absorbance at the concentration of 10 µg/mL CA, but no change in absorbance of more than 5 % was observed within 24 h (Table-3).

TABLE-3
STABILITY OF 10 µg/mL CA-AN DERIVATIVE

Time (h)	Absorbance (376 nm)
0	0.448
0.5	0.448
1.0	0.448
2.0	0.449
3.0	0.448
4.0	0.447
5.0	0.448
6.0	0.448
12.0	0.447
16.0	0.447
24.0	0.445
32.0	0.445
48.0	0.443

Calibration plot: The effect of variation in the concentration of CA on absorbance was studied. A linear calibration curve was obtained which obeyed the Beer's law within the ethanolic concentration range 5-25 µg/mL of CA with coefficient of determination r^2 0.9998 (Fig. 6). The validity of the calibration curve was obtained by the analysis of test solution of CA and the percent relative error was found within ± 1-4 %.

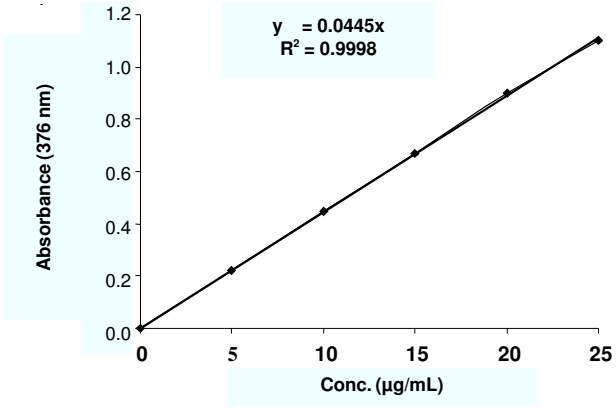


Fig. 6. Calibration curve of cinnamaldehyde (0.001 %) using AN as derivatizing reagent

The cinnamon barks available in the market as an unbranded and the cinnamon srilankan were analyzed as crude drugs for the determination of CA and amounts found were 0.40-0.57 % with (RSD) 0.01-0.03 % (Table-4). The results obtained were compared with the values obtained by measuring the absorbance of pure CA at 286 nm without derivatization and a good correlation was obtained.

TABLE-4
ANALYSIS OF CINNAMALDEHYDE FROM CINNAMON BARKS, CINNAMON OILS AND HERBAL PREPARATIONS USING AN AS DERIVATIZING REAGENT

Name of brand	Amount found (%) (relative standard deviation %)
Mixed cinnamon bark	0.400 (0.010)
Srilanka cinnamon bark	0.570 (0.030)
Cinnamon oil. Naedern Holland	46.000 (1.520)
HQ oil, Karachi	0.580 (0.070)
Cinnamon oil, Lahore	0.440 (0.020)
Carmizol	0.020 (0.015)
Gastomy	0.060 (0.019)
Gastokil	0.010 (0.010)
Kisirin	0.320 (0.010)
Gasto	0.020 (1.990)
Gasnil	0.003 (1.800)
Gastofill	0.010 (0.890)
Gastovena	0.270 (1.230)
Gasto	0.020 (1.890)
Gaskil	0.340 (0.050)

The cinnamon oils Naedern Holland b.v., Haque planters and cinnamon oil from standard flavor company were analyzed as unani remedy preparations and the amounts found were within 0.44-46.00 % with (RSD) 0.02-1.57 %. The Syrups namely Carmizol, Gastomy, Gastokil, Kisirin, Gasnil, Gasto, Gastofill, Gastol, Gastovena and Gaskil containing extract of cinnamon bark were analyzed as herbal preparations for their contents of CA and the results indicated the amounts within 0.003-0.340 % (RSD) 0.80-1.23 % (n = 3) (Table-4).

However the cinnamon oils and syrups could not be analyzed by measuring the absorbance of CA at 286 nm, because of interfering effects of excipients. The derivatization with AN resulted in the bathochromic shift in the wavelength to 376 nm, at which the excipients did not interfere the determination of CA in oils and syrups.

The mean values (%) and range of error % at (95 % confidence limit) of three brands of oil samples, two brands of bark samples and ten brands herbal samples were 15.67 ± 10.11, 0.49 ± 0.06 and 0.10 ± 0.02, respectively (Table-5).

TABLE-5
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY

Parameter(s)	Values
Wave length λ_{max} of CA-AN derivative (nm)	376
Beer's law limits (µg/mL) of CA-AN derivative	05-25
Molar absorptivity (L mol/cm)	5.8×10^3
Sandells sensitivity (µg/mL 0.004 absorbance unit)	2
Regression equation (y)[a]	
Slope (b)	0.448
Intercept (a)	0
Coefficient of determination	0.9998
Relative standard deviation (%)	0.003 - 1.527
Mean values (%) of three brands of oil samples ± range of error % at (95 % confidence limit)	15.67 ± 10.11
Mean values (%) of two brands of bark samples ± range of error % at (95 % confidence limit)	0.49 ± 0.06
Mean values (%) of ten brands herbal samples ± range of error % at (95 % confidence limit)	0.10 ± 0.02

Conclusion

The developed method is accurate, sensitive, precise and inexpensive. Although the molar absorptivity of the developed method is less than UV reference method, but the λ_{max} is considerably higher than the reference method. This is an advantageous quality of the developed method which is used to avoid the interferences from associated materials which may absorb in the UV region. The method was used for the determination of CA contents from cinnamon crude drugs, cinnamon herbal oils and herbal preparations.

REFERENCES

1. M. Heinrich, J. Barnes and S. Gibbons, Fundamentals of Pharmacognosy and Phytotherapy, Churchill Livingstone, Elsevier Ltd., Spain, p. 71 (2004).
2. M.M. Beg, R.P.S. Chauhan and I.C. Shukla, *Proceed. Indian Nat. Sci. Acad. A*, **50**, 93 (1980).
3. A.A. Seif Eldin, M.A. Korany and N.A. Abdel-Slam, *Anal. Lett*., **16**, 891 (1983).
4. K. Zaitsu and Y. Ohkura, *J. Chem. Pharm. Bull*., **23**, 1057 (1975).
5. T. Shan-Yuan and C. Sheng-Chih, *J. Nat. Prod*., **47**, 536 (1984).
6. M.J. Cikalo, S.K. Poole and C.F. Poole, *J. Planar Chromatogr. Modern TLC*, **5**, 135 (1992).
7. S.K. Polle and C.F. Polle, *Analyst*, **119**, 113 (1994).
8. A.W. Archer, *J. Chromatogr. B*, **447**, 272 (1988).
9. C. Villa, R. Gambaro, E. Mariani and S. Dorato, *J. Pharm. Biomed. Anal*., **44**, 755 (2007).
10. K. Sagara, T. Oshima, T. Yoshida, Y. Tong, G. Zhang and Y. Chen, *J. Chromatogr. B*, **409**, 365 (198).
11. K. Kanemaru, T. Takaya and T. Miyamoto, *Nippon Shokuhin Kogyo Gak-Kaishi*, **37**, 565 (1990).
12. Y.C. Lee, C.Y. Huang, K.C. Wen and T.T. Suen, *J. Chromatogr. A*, **2**, 137 (1995).
13. D. Ehlers, S. Hilmer and S. Bartholomae, *Lebensm Unters Forsch*, **200**, 282 (1995).
14. D. Ronghua, L. Kang, L. Qing and B. Kaishun, *J. Chromatogr. Sci*., **42**, 207 (2004).
15. M. Katayama, Y. Mukai and Taniguchi, *Analyst*, **115**, 9 (1990).
16. D. Frank, D. Christophe, J. Daniel, C. Alain and S. Pat, *J. Sep. Sci*., **29**, 1587 (2006).
17. W. Chen, *Yauxue Tongban*, **22**, 612 (1987).
18. W.M. Blakemore and H.C. Thompson, *J. Agric. Food Chem*., **31**, 1047 (1983).
19. Y. Nakzawa, T. Sachie, M. Izumitni and C. Inagaki, *Rakuno Kagaku Shokuhin no kenlcu*, **30**, A39 (1981).
20. Y. Bing-Sheng, L.A.I. Shu-Guang and T.A.N. Qiao-Lin, *J. Chem. Pharm. Bull*., **54**, 114 (2006).
21. F. Mendel, K. Nobuyuki and A.H. Leslie, *J. Agric. Food Chem*., **48**, 5702 (2000).
22. M.Y. Khuhawar and F.M.A. Rind, *J. Chromatogr. B*, **766**, 357 (2002).