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Validation of analytical method for determination of querctin concentration in cane sugar and cane juice

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ABSTRACT

A rapid and sensitive and reliable method was developed for the determination of querctin concentration at different pH of cane sugar and cane juice. The validation of analytical method for the determination of querctin concentration was done by HPLC. The method consisted of extracting with methanol from cane sugar. The extracts were subjected onto column filled with florisil column chromatography. The extraction of querctin was from cane juice with ethyl acetate. Quantification is performed by reversed phase HPLC with UV detector.

KEYWORDS

Cane sugar;
Cane juice;
Column chromatography;
HPLC.

INTRODUCTION

Many efforts have been made in the past 25 years or so to isolate and identify sugar colorants. However, only a few papers report the identification of colorants in sugars. Sugar colorants first of all separated from cane sugar by high voltage paper electrophoresis method[1]. These color pigments were described in terms of their color, fluorescent color and mobility on high voltage paper electrophoresis. These are chlorogenic acid, caffeic acid, p-hydroxy cinnamic acid, kaempferol and umbeliferone, 4-hydroxy – 3- methoxy cinnamic acid and 4-hydroxy -3, 5-dimethoxy cinnamic acid. Tricin glycosides, Iso-orientin and its glycosides and isovitexin have been identified from the leaves of a few selected Saccharum species[2]. A method was developed for the isolation and fractionation of colorants from sugar factory products[3]. It was applicable even to low color products such as crystalline sugar. The isolated colorants was fractionated by high performance gel permeation chromatography and reverse phase HPLC and detected by UV absorbance. A selected method is available in scientific literature for removal of phenolic compounds from soft sugars[4]. The polystyrene resins XAD-2 and XAD-4 were used as they enabled 74% recovery. Gas chromatography of XAD-2 and XAD-4 extracts of sugars revealed a large number of peaks.

Recently HPLC technique was suitable for separating flavonoids and phenolic acids[5]. The samples analyzed were raw liquor, fine liquor and 3rd boiling granulated sugar. HPLC study showed that numerous flavonoids were present in the liquor at ppm levels. Traces of flavonoids were also detected in refined sugars. However, a new flavonoid existed in cane sugar by ion exchange column chromatography on XAD-2 resins[6]. From the coloring matter were isolated seven flavonoid C-and O-glycosides two of which have not previously been reported in cane sugar. Their structures were established by spectroscopic and chemical evi-

dence. Although presence of coloring matter in sucrose crystals and classification according to their mechanism of formation i.e. non enzymatic reactions independent of oxygen (Melanoidins and Caramels) and those formed in presence of oxygen (polyphenols) were also studied[7]. The inclusion of colorants within the sucrose crystals from certain phases related to growth rates of these phases.

Because trace amount of five classes of flavonoids are: anthocyanins, catechins, chalcones, flavonols and flavones present in sugar cane so usually detected by HPLC techniques[8]. These colorants were adsorbed efficiently by resins. In addition separation of flavonoid colorants in cane refinery liquors by HPLC; 13 flavonoids were found to be reported[9]. The predominant flavonoids in fine liquor were Apigenin 6, 8 di-C-glycosides, isoorientin 7, 3-dimethyl ether and tricin 7-O-glycosides. Preparation of three types of colorant samples (melanoidins, alkanline degradation products of hexose's and caramel) having solutions (15^0 brix, pH-8) passes through ion exchange resin columns; a cationic resin removed about 90% melanoidins but<10% of the other two colorants whereas the anionic resin removed about the 90% of the degradation products of hexose's but<10% of other colorants[11]. In cane products the-colorants were mainly caramels. Sugarcane flavonoids consist of a complex mixture of aglycones and glycosides which is present in sugar, sugarcane leaves and sugarcane juice a HPLC-UV method was used for their quantification[12,13]. Identification and quantification of phenolic compounds in sugar cane (Saccharum officinarum) juice by analytical HPLC method, showing the predominance of flavones (apigenin, luteolin and tricin derivatives)[14]. Micro fractionation of flavones in sugarcane and juice and identified by HPLC-UV analysis combined with HPLC micro fractionation monitored with HPLC by TLC using β-carotene as detection reagent is also a useful technique[15].

MATERIAL AND METHODS

Analytical reference standards of Quercetin (98.5% purity) obtained from sigma Aldrich Limited Mumbai, India. All the other chemical and solvents were used in the study analytical and HPLC grade.

Fortification of samples

A representative samples 50g different sugar samples viz. S, M, L grade were transferred in 250 ml Erlenmeyer flask. The sugar samples were fortified with Quercetin at two different fortification LOQ and 1O X LOQ Lpveis, separately. A Volume of 0.5 and 5.00 ml Quercetin was transferred to each conical flask for 0.02 and 0.20 ppm fortification levels. The control samples were processed similarly after 0.5 and 5ml methanol was added.

Extractions and sample clean up

A volume of 100 ml methanol was added into the Erlenmeyer flask at different fortified sugar samples were placed onto orbital shaker for 30 minutes, after shaking, the solutions were filtered into the round bottom flask of 500 ml capacity through what man filter paper No. 1. The residual cake was extracted twice. The methanol extract were collected, pooled and concentrated to dryness using vacuum evaporator at 40°C. The residue re-dissolves in methanol. The concentrated soil samples were cleaned by column chromatography. A glass column was packed with adsorbent in between two layer of anhydrous sodium sulphate the cleanup of sugar samples process florisil was use as adsorbent column was pre- conditioned with methanol and concentrated extract were loaded onto top of the column and eluted with 100 ml acetoriitrile. Elute were concentrated a using rotary vacuum evaporator and residue re-dissolve in 5m1 acetonitrile and final volume was made up to the mark with acetonitrile (l0ml).

Extraction of juice samples

The fortified cane juice samples were extracted with ethyl acetate. After extraction the samples are filtered and evaporated to the dryness using a rotary vacuum evaporator. The residue re-dissolves in 5m1 acetonitrile and final volume was made up to the mark with acetoninile (l0mi). The analysis of Quercetin in sugar and juice using reverse phase HPLC technique was used for quantitative analysis. A Shimadzu LC-2010 AHT with LC-solution softer ware, C18 column, Phenomena, 25cm length x 4.6 mm i.d. and 0.5 mu particle size, Mobilç phase A: 0.01% (v/v) acetic acid in water (60). Add 0.1 ml acetic acid and dilute to 1 liter with water, mobile phase B: acetonitrile (40)

at 1ml flow rate and detector set a 252 nm 2.rnax was used for analysis. Quercetin standard showed sharp peak at 4.93 minute under the described HPLC condition.

RESULTS AND DISCUSSION

The linearity of the detector response was tested for Quercetin, in solvent and in matrix over the range of 0.02 to 5.00 mg/kg. A very precise linear relation between the injected amount and the resulting peak area was observed over the entire range with correlation coefficients between 0,999. (Ishii-Y et. al,) has also reported HPLC method developed for the determination of Quercetin residue in 2 kinds of sugar and juice. The method consisted of extracting with acetonitrile / water (80:20 v/v), pre washing of the concentrated extracts with cyclohexane and alkaline solution, silica gel column chromatography, and finally reversed-phase HPLC. The recoveries of Quercetin were 75-109%. The limits of determination of the method were 0.005, 0.01 and 0.02 mg/kg for sugar, juice respectively. The accuracy and precision of the method was evaluated on the basis of the recoveries obtained for fortified samples sugar and cane juice, the limit of quantization (LOQ) was 0.02 mg/kg for Quercetin in sugar and 0.02 mg/L in juice. The limit of detection (LOD) was 0.01 mg/kg for Quercetin in sugar and 0.01 mg/L in juice. Recoveries for Quercetin were 95.18, 94.66, 95.27 and 94.78 % in S, L, M grade sugar. The recoveries for Quercetin were 96.86, 96.14 and 92.34 % in juice pH4, pH7 and pH9. The method for Quercetin recovery was also reported (Ralf et. al.) The repeatability of the metho4 was determined for each analyze by running a set of five recoveries each at two different fortification levels for selected matrices. The resulting mean recovery rates ranged from 79 to l04% with relative standard deviations between 0.8 and 15.3%. These data demonstrate the excellent sensitivity, selectivity and precision of the method. Similar results were found in present method validation for different soil and waters. The repeatability of the method was determined for each fortification levels by running a set of five recoveries each different fortification levels for selected matrices. The % RSD was the resulting mean recovery rates ranged from 94.66 to 95.27% in soil with relative standard deviants between 1.21 to 3.37%. The % RSD was the resulting mean recovery rates ranged from 92.34 to 96.86% in water with relative standard deviations between 1.66 and 3.23%. These data demonstrate the excellent sensitivity, selectivity and precision of the method.

TABLE 1 : Accuracy (% Recovery) and precision (%RSD) of quercetin in sugar

Substrates	Fortification of LOQ and 10 x LOQ lives in mg/kg	% Recovery	Mean % Recovery	SD	% RSD
S grade sugar	0.02	97.20	95.18	1.18	12.21
	0.20	93.16		2.24	2.50
L grade sugar	0.02	97.25	94.66	1.86	1.91
	0.20	92.07		3.10	1.91
M grade sugar	0.02	97.00	95.27	1.97	2.03
	0.20	93.54		3.01	23.22

TABLE 2 : Accuracy (% Recovery) and precision (%RSD) of quercetin in juice

Substrates	Fortification of LOQ and 10 x LOQ lives in mg/kg	% Recovery	Mean % Recovery	SD	% RSD
Juice pH 4	0.02	97.60	96.86	1.63	1.67
	0.20	96.11		2.21	2.30
Juice pH7	0.02	96.30	96.14	1.60	1.66
	0.20	92.07		3.10	3.23
Juice pH9	0.02	95.00	95.27	1.97	2.03
	0.20	93.54		3.01	23.22

REFERENCES

[1] L.Farber, F.G.Carpenter; Proc.14th Congr.ISSCT, 1589-1600 **(1971)**.

[2] C.A.Williams, J.B.Harborne; Phytochemistry, **13**, 1141-1149 **(1974)**.

[3] H.T.Cheng, C.R.Wang; Rep.Taiwan Sugar Res. Inst., **98**, 47-58 **(1982)**.

[4] M.A.Godshall, F.J.Roberts; Proc.of the Sugar Processing Res.Conf., 47-72 **(1982)**.

[5] **(a)** N.H.Paton, P.Smith; Proc.of the Sugar Processing Res.Conf., **1-23**, 139-145 **(1983)**; **(b)** Int.Sugar J., **85**, 102-105 **(1983)**.

[6] T.H.Mabry, Y.L.Liu, J.Pearce et. al.; J.Natural Products, **47**, 127-130 **(1984)**.

[7] G.Mantovani, G.Vaccari, G.Sgualdino et al.; Ind.Sac. Ital, **78**, 7-14 **(1985)**.

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[8] P.Smith, N.H.Paton; Sugar Technol.Rev., **12**, 117-142 **(1985)**.
[9] N.H.Paton, P.Smith, T.J.Mabry; Int.Sugar J., **87**, 1043 **(1985)**.
[10] V.Maurandi; Ind.Sac.Ital., **81**, 47-52 **(1988)**.
[11] Sucrerie Maghrebive; (ISERF), Rabat, Morocco, **44**, 3-7 **(1990)**.
[12] R.Colombo, J.H.Yariwake, E.F.Quciroz, Karine Ndjoko, K.Hostettmann; J.Chromatography A, **1082(51-59)**, **(2005)**.
[13] R.Colombo, F.M.Lancas, J.H.Yariwake; J.Chromatography A, **1103(1)**, 118-124 **(2006)**.
[14] M.D.Almeida, J.Novoa, A.V.Linars, A.F.Lajolo, F.M.Ines Genovese; (PFHN), **61**, 187-192 **(2006)**.
[15] Fabiana C.Vila, R.Colombo, O.Tatiana, De Lira, J.H.Yariwake; J.Braz., Chem.Soc., **19(5)**, 903-908 **(2008)**.
[16] T.J.Mabry, K.R.Markham, M.B.Thomas; 'The Systematic Identification of Flavonoids' Springer - Verlag, New York, 126 **(1970)**.
[17] T.J.Mabry, K.R.Markham, M.B.Thomas; 'The Systematic Identification of Flavonoids', Springer - Verlag, New York, 294 **(1970)**.