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Research Article

ESTIMATION OF PRESERVATIVE FROM LOCAL MARKET BEVERAGES OF AMRAVATI (M.S.) BY HPLC TECHNIQUE

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ABSTRACT

Identification and quantification of benzoic acid from local market beverages of Amravati (M.S.) was investigated at HPLC Shimadzu LCAT system. The HPLC analysis was performed by isocratic elution with mobile phase acetonitrile and ammonium acetate buffer 4.2 P^H in 40:60 respectively. UV detection was carried out at 228nm by using phenomenex C_{18} column (250 × 4.6 mm, 5µ) at ambient temperature and flow rate of 1ml/min by external standard methods. Precision, Accuracy, Robustness, Stability, Sensitivity LOD and LOQ were established.

Keywords: Benzoic acid, HPLC, Beverages.

INTRODUCTION

Benzoic acid extensively used as preserving agent in food, fruit juice, soft drinks, beverages, tooth pastes, mouth washes, dentifrices, cosmetic and pharmaceuticals. It is generally effective in controlling mould and inhibiting yeast growth and also in preventing a wide range of bacteria aggressions. Although this prevents delays of nutritional losses due to microbiological, enzymatic or chemical changes of food during its shelf life, it is harmful at higher than permitted safety level. According to “A Consumer Direction of food additives” the average level of benzoic acid found in food is between 0.05 and 0.1 percent. UV Spectroscopic determination of benzoic acid in refined pthallic anhydride and determination on self associate 5-caffeoylquinic acid and its complexation with sodium hydroxide and many simultaneous estimations have been reported by UV Spectroscopic techniques[1-3]. Fluorescence and energy transfer process of humic substance and related model compounds in terbium complexes can be studied and also for determination of amines this technique is used[4-5]. X-ray diffraction and thermal analysis studies of β–cyclodextrin-para–aminobenzoic acid, salicylic acid derivatives, II comparison of 2- hydroxyl and 2, 4 and 2, 5- dihydroxy derivatives and many monomer and dimer analysis have been reported by IR spectrophotometry technique[6-8]. Simultaneous determination of procaine and benzoic acid by derivative spectrophotometry is used[9, 10]. TLC technique can be used to estimate benzoic acid in pharmaceutical analysis and organic acid analysis[11,19]. There are reports on use of gas chromatography for determination of benzoic and sorbic acid level in orange, tomato concentrate and serum or plasma[12, 13]. Spectral identification and HPLC quantification of benzoic acid from natural juices, Brazilian food, soft drinks and many food stuff has been also reported[14-18]. During last 2-3 decades increasing consumption of soft drinks causes alarming health problems. So the estimation of the class II preservative i.e. Benzoic acid in the soft drink in local market of Amravati major concern of the research in this study.

MATERIALS AND METHODS

High performance liquid chromatography system with LC solution data handling system (SHIMADZU-LCAT) was used for analysis. The system was controlled and data was recorded with spinchrome (RF software). The assays were performed on LC system consisting of SHIMADZU-LC 20 AT pump and SHIMADZU-UV detector. The injection volume was 20µl and it is injected in rhenodyne injector system. The detector was set at 228nm and peak area was integrated automatically by computer using spinchrome

CRF software. Detection was carried out by using phenomenex C_{18} column (250mm × 4.6mm, 5µ (micron)) at ambient temperature all the calculation consisting quantitative analysis were perform with external standardization by the measurement of peak area.

Benzoic acid, HPLC grade acetonitrile and water procured from fisher scientific pune and of analytical grade. Soft drinks were purchased from local commercial centers of Amravati city (M.S.)

Preparation of mobile phase

Acetonitrile and ammonium acetate buffer solution are used as mobile phase. Ammonium acetate buffer solution was prepared by dissolving 0.4 gm ammonium acetate in 1 liter HPLC grade water. P^H of this buffer solution was adjusted to 4.2 by acetic acid. Mobile phase was filter through 0.45 µ filter paper and degassed.

Preparation of standard solution

Standard stock solution of concentration 1mg/ml of benzoic acid was prepared using mobile phase acetonitrile and buffer ammonium acetate having P^H 4.2 (40:60). From the standard stock solution working standard solution were prepared using mobile phase to get 0.005-0.025 µg/ml. These solutions were filter through 0.25 µ membrane filter and degassed.

Preparation of sample solution

Solutions of the sample soft drinks were prepared and centrifuged at appropriate rpm then filtered through 0.25µ membrane filter and degassed. All solutions were freshly prepared before the analysis.

RESULT AND DISCUSSION

Method optimization

HPLC analysis was performed by isocratic elution with a series of mobile phase containing acetonitrile and buffer having P^H 4.2. The best results were obtained with the use of mobile phase acetonitrile and buffer having P^H 4.2 (40:60 v/v). The flow rate was determined by testing the effects of flow rate on a pick area and resolution i.e 0.9ml/min, 1ml/min, 1.1ml/min. 1ml/min was found to be optimum. The suitable wavelength selected for monitoring the benzoic acid was 228nm. 20µl standard solution was injected for HPLC analysis and peak area was recorded at 228nm.The chromatographic run time was 5 min and column void volume 3.180 min. (Table no1) Through the study the stability of chromatograph i.e system was monitored by calculating the resolution, selectivity and peak summery.

Accuracy

To determine the accuracy, prepared standard solutions were injected six times as attest sample. The result in accuracy is given in table no 2. Revealed that the method is accurate.

Linearity

Linearity of the method was studied by injecting five concentration of the benzoic acid prepared in the mobile phase in triplicate in to LC system, keeping the injection volume constant. The peak areas are plotted against corresponding concentration to obtain calibration graph. The slope and intercept value for calibration curve is Y= 2140.33714*x+2.73629 which shows the regression equation correlation coefficient (r^2) RSD values of the slope and intercept for benzoic acid. Excellent linearity is obtained for compounds between the peak area and concentration of 0.005-0.025mg/ml with r^2= 0.9909455. Fig (1) shows the calibration curve of standard solutions of different concentrations.

LOD and LOQ

By using method based on standard deviation of response and slope for benzoic acid the LOD was found to be 0.0087 mg/l and LOQ was 0.026µg/ml. Table no 3

Ruggedness

The ruggedness of HPLC method was evaluated by carried out analysis using standard working solution; the same chromatographic system and same column at different days. The comparable detector responses obtain on different day indicated that the method is capable of producing result with high precision and fairly rugged. Table no 4 and 5

Robustness

To differentiate, the results of 0.005mg/ml benzoic acid under various conditions were taken. Each mean value was compared with mean value obtained by optimum condition. The statically comparison was done with the test and no difference was found between results. Therefore, the method is robust to the small change in experimental condition. Table no 6.

Stability

Stability of standard solution of benzoic acid was evaluated under different storage conditions. For short term stability of standard solution it was kept at normal room temperature for 24 hours and then kept in refrigerator at 4° c for 15 days. The stability results were evaluated by comparing peak area ratios for benzoic acid with those of freshly prepared solution. The results found within 90-91% of initial values indicate that benzoic acid can be considered stable under the conditions investigated.

Specificity

The excipients used in different formulation product did not interfere with the benzoic acid peak and thus the method is specific for benzoic acid. For further confirmation of the method UV scan of spiked benzoic acid is taken in range 200-400nm and no significant change was found. By comparing the absorbance of pure benzoic acid and spiked benzoic acid at the analytical wavelength of benzoic acid.

Estimation of benzoic acid in commercial soft drinks

Appropriate solutions of commercially available soft drinks in Amravati (M.S.) like Fanta, QRC Lemon, RC Cola, Frooti, Appy-fiz, Limca, and Amla were prepared in selected mobile phase in varied concentration.

By working on different sample solutions the graph was plotted for amount against retention time of different sample solutions which is shown in Fig (2). Concentration of benzoic acid in different sample are tabulated in table no 7.

CONCLUSION

This contribution purpose a simple selective and rather fast HPLC method for determination of benzoic acid in soft drinks commercial samples. The method also demonstrated its applicability for the study of large number of soft drink samples with complex matrix. The obtained results showed that the benzoic acid concentration varied between the different kinds of beverage samples with level lower than the maximum values established by national and international legislation (table no:- 7). This database can help the consumer in the selection of more adequate products for their health.

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Table 1: Method optimization

Parameter	Optimum condition
Chromatographic column	SHIMADZU-HPLC, phenomenex C_{18}, 250×4.6mm, 5µ
Mobile phase	Acetonotrile : Ammonium acetate buffer having P^H 4.2 (40:60)
Flow rate	1ml/min
Detection	228nm
Injection volume	20 µl
Temperature	Ambient
Retention time benzoic acid	3.180

Table 2: Accuracy developed method

Compound	Spiked concentration	Measured concentration	RSD	Deviation
Benzoic acid	5 mg/l	4.7mg/l	0.332	0.94%

Table 3: Linearity result, limit of detection (LOD) and limit of quantification (LOQ)

Compound	λ value	Equation	r^2	LOQ µg/ml	LOD µg/ml
Benzoic acid	228	Y=2140.33 X+2.7336	0.9909455	0.000026	0.0000087

Table 4: Day to day variability according to area

Date	14 March	15 March	28 March
Name	Benzoic acid	Benzoic acid	Benzoic acid
Area	39.777	39.799	33.781
SD	0.133	0.0666	0.233
%RSD	0.33	1.67	0.68

Table 5: Day to day variability according to retention time

Date	14 March	15 March	28 March
Name	Benzoic acid	Benzoic acid	Benzoic acid
RT	3.180	3.175	3.168
SD	0.333	0.166	0.133
%RSD	10.45	5.24	4.2

Table 6: Robustness of method

Flow rate	0.9ml/min	1ml/min	1.1ml/min
Mean	3.640	3.267	3.013
SD	0.066	0.133	0.1
%RSD	0.0182	0.040	0.0331

Table 7: Amount of benzoic acid in local market beverages

Sample	RT(min)	Response	Amount (mg/l)	Compound
Limca	2.980	2.365	0.0014	Benzoic acid
Appy- fizz	3.247	57.339	0.0360	Benzoic acid
Fanta	3.200	120.994	0.0768	Benzoic acid
Frooty	3.183	71.829	0.0395	Benzoic acid
QRC Lemon	3.013	2.388	0.1003	Benzoic acid
RC Cola	3.035	28.222	0.0177	Benzoic acid
Amla	0	0	0	Benzoic acid

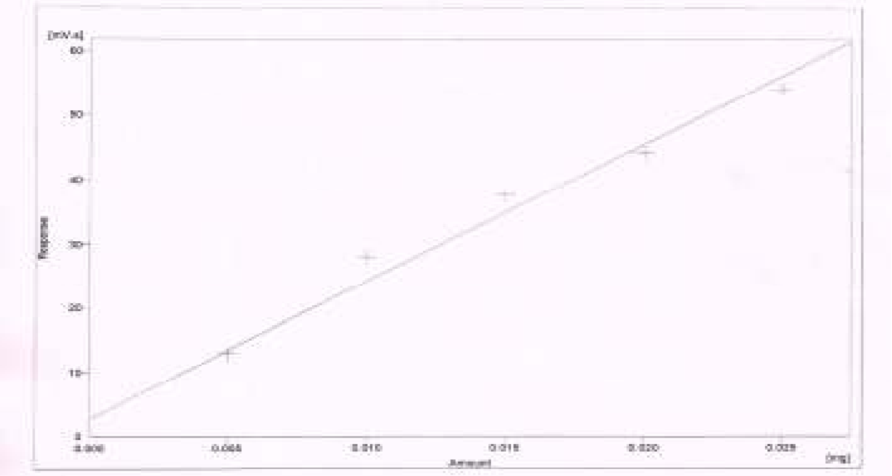

Fig. 1: Calibration curve of standard solution

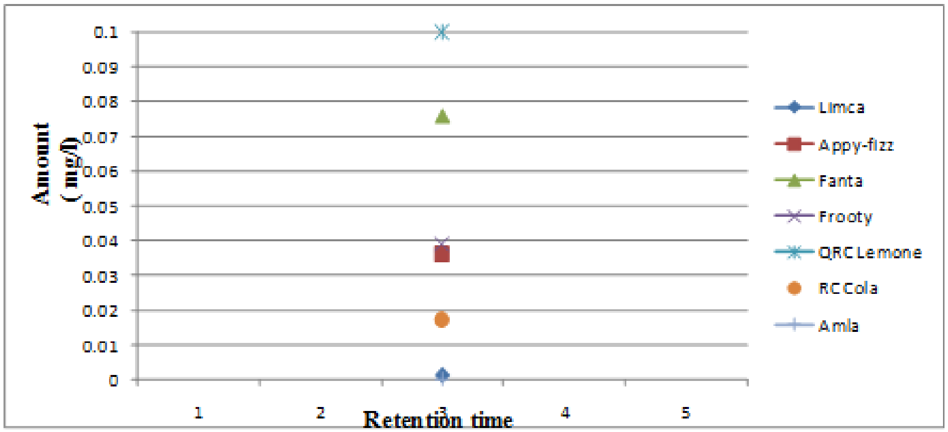

Fig. 2: Graph of sample solutions

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