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Short communication

Determination of diazepam in cold drinks by high-performance thin-layer chromatography

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Abstract

A methodology was developed for the detection and quantitation of diazepam in non-alcoholic carbonated beverages and fruit drinks which are adulterated for criminal motives. The extraction of diazepam from the five brands of spiked and simulated cold drinks was carried out at pH 8.5 by two different extraction solvents viz., diethylether and chloroform. The identification of diazepam was done on the basis of R_F values and in situ UV spectra. The quantitation was carried out by densitometric scanning of the chromatogram at a wavelength of 230 nm. The method is rapid and reliable for qualitative and quantitative analysis of cold drinks adulterated with diazepam and can be used by law enforcement laboratories for routine analysis.

Keywords: Cold drinks; Diazepam

1. Introduction

Diazepam, a tranquillizer and controlled drug, is easily available in the market at low price. This drug finds ample scope for its misuse by anti-social elements. The use of diazepam for criminal motives is in two ways, the addition of diazepam in cold drinks (non-alcoholic beverages and fruit drinks) in order to make people addict to a particular brand, secondly it may be used as sedative in liquid foods, like juices, milk, coffee, tea, etc., for the purposes of cheating or deceiving a person. In recent past the instances of addition of diazepam in cold drinks have been reported by law enforcement agencies. It, therefore necessitated to develop a simple and rapid method for the qualitative and quantitative analysis of diazepam in cold drinks. The study of the literature reveals that various analytical methods like gas chromatography (GC) [1–3], GC–mass spectrometry (MS) [4,5], high-performance liquid chromatography (HPLC) [6–8] and spectrophotometry [9–11] are available for the determination of diazepam and other benzodiazepines in various matrices. This indicates that virtually all the quantitative estimations of diazepam have been reported in biological fluids, viscera and alcoholic drinks but scant attention has been paid by the researchers on the analysis of benzodiazepines in food drinks. Therefore, it is considered worthwhile to develop a high-performance thin-layer chromatography (HPTLC) method for the detection and determination of diazepam in cold drinks which can be used

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for routine examinations of such type of crime exhibits.

2. Experimental

2.1. Materials and instruments

Diazepam tablets (brand name valium) and five brands of cold drinks were procured from the local market for simulation and recovery studies. The cold drinks include two carbonated citrus flavor-based beverage (Fanta and Limca), two carbonated caffine-based beverage (Coke and Thumps up), and one mango fruit-based cold drink (Maza). All solvents used in the experiments were of chromatographic/analytical-reagent grade. Merck 20×10 cm TLC plates pre-coated with silica gel 60 F 254 were used. Plates were pre-washed with methanol and dried before experiments.

The HPTLC system (Camag) consisted of a Linomat IV sample applicator equipped with 100-μl syringe and a scanner II operated using Camag software CATS 3 loaded on personal computer 386.

2.2. Preparation of standard solutions

A stock solution of diazepam, 0.20 mg/ml in concentration was prepared in ethanol. This alcoholic solution of diazepam was used for spiking the cold drinks for the recovery studies. Various calibration standards 20, 30, 40, 50, 80 μg/ml were prepared from stock solution for constructing the calibration curve.

2.3. Preparation of spiked and simulated sample

One carbonated caffine-based (Coke) and one mango fruit-based (Maza) cold drink were chosen for spiking and recovery studies. Five samples of each of the above cold drinks were spiked by adding 2.5 ml of standard stock solution of diazepam in a 25-ml volumetric flask and making up its final volume with the cold drinks.

For simulation of five brands of cold drinks pharmaceutical grade diazepam tablets were powdered and assayed by standard method [12]. An accurately weighed portion of this powder equivalent to 0.50 mg of diazepam was transferred into a 25-ml volumetric flask containing 2 ml of ethanol. After thorough shaking it was mixed with cold drink making up its final volume. The process followed for simulations was similar to that of generally adopted in crime practices.

2.4. Extraction

All the spiked and simulated samples were neutralized and their pH was adjusted to 8.5 with sodium hydroxide and 0.5 *M* disodium hydrogenphosphate. The above samples (35 ml after adjusting pH) were extracted five times with 20-ml portions of chloroform for 120 s by shaking gently. Another set of spiked and simulated samples was extracted similarly five times with 20-ml portions of diethyl ether. Organic phases were collected separately. Both chloroform and diethyl ether extracts were evaporated to dryness on water bath. Residues were reconstituted in ethanol to a fixed volume.

2.5. Chromatography

Standard solutions of diazepam, extracts of spiked, simulated and blank samples of cold drinks were applied on pre-coated, and pre-washed TLC plates, with the help of applicator Linomat IV at the rate of 7 s/μl in the form of a 6-mm band with in-between space of 4 mm. The plates were developed in paper lined twin trough chamber saturated for 20 min with 10 ml solvent system of chloroform–acetone (85:15). The plates were developed up to a distance of about 5 cm and dried with a drier.

2.6. Scanning

The plates were scanned by the scanner II at the wavelength of 230 nm in reflectance/absorbance mode keeping slit width 4 mm, slit length 6 mm and scanning speed of 4 mm/s. The densitograms were further scanned for their in situ UV spectra from 400 to 200 nm.

3. Results and discussion

Some of the densitograms of standards, simulated

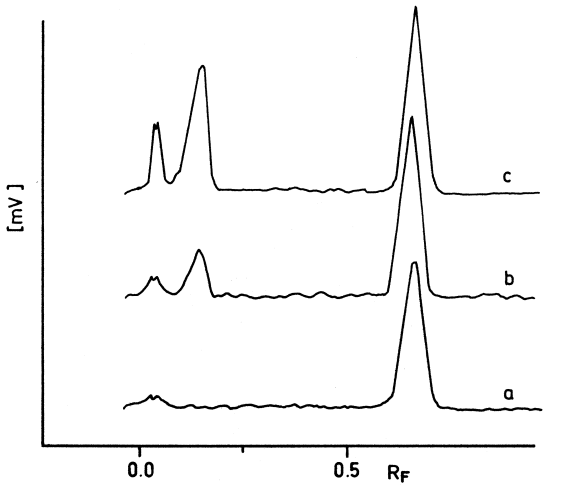

Fig. 1. Densitogram of (a) standard diazepam (200 μg/6 mm band). (b) Chloroform extract of Coke sample spiked with diazepam. (c) Chloroform extract of the Coke sample simulated with diazepam.

and spiked samples obtained after the densitometric scanning are shown in Fig. 1. On evaluation of chromatogram the standard, simulated and spiked samples, it was observed that all the samples gave peak at the R_F value of standard applied on the same plate. This indicated the positive identification of diazepam. The detection of diazepam was further confirmed by in situ UV spectra of the above spots/bands of the chromatogram (Fig. 2). On evaluation of the in situ UV spectra, it was observed that the similar type of spectra were obtained in all spiked and simulated samples which were found to be identical with standard diazepam. Blank samples did not give peak/spot at the R_F value corresponding to that of standard diazepam.

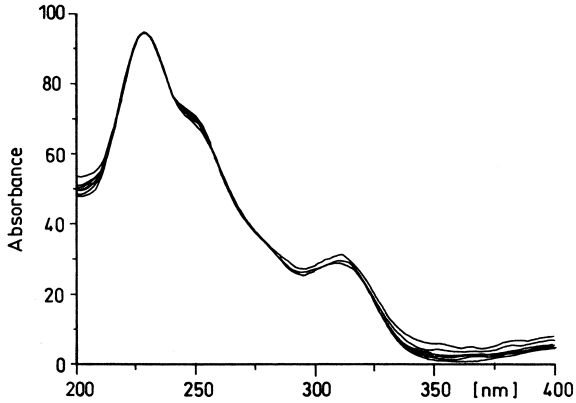

Fig. 2. In situ UV spectra of diazepam extracted from cold drinks.

Within plate the difference in R_F values was found to be 0.02 (minimum value) and 0.04 (maximum value). Among the plates variation in R_F values was from 0.61 to 0.67. The variation in the λ_{max} value of in situ UV spectra was in the range of 227–230 nm within the plate and 226–230 nm among plates. The limit of detection (LOD) of the diazepam was found to be 30 ng per 6-mm band for obtaining a smooth spectrum which is comparable to that of spectrum of standard diazepam on each plate.

Quantitative analysis of diazepam in all the simulated and spiked samples was done on the basis of peak area in linear regression mode. The calibration range was set from 200–800 ng, which showed acceptable linearity. The calibration curves were constructed for each plate in order to determine the concentration of diazepam in the extracts of cold drinks. The two replicate of each standard 20, 30, 40, 50 and 80 μg/ml were plotted against the peak area. The value of correlation coefficient (r) among the plates varied from 0.9914 to 0.9998 and value of intercept (a) and slope (b) varied from −1.17 to 9.60 and 0.96 to 2.41, respectively. Linear regression equation for the reference standard taken on some of the plates along with extracts of simulated and spiked samples of cold drinks, are represented as, $y=3.04\ (\pm 2.67)+1.47\ (\pm 0.22)x$, ($n=5$, $r^2=0.9997$) and $y=3.75\ (\pm 1.29)+1.23\ (\pm 0.12)x$, ($n=4$, $r^2=0.9916$), respectively.

Recovery data of spiked samples (Table 1) indicated higher recoveries of diazepam in both the cold drinks with chloroform extraction. While low recoveries were obtained in the cold drinks with ether extraction. The relative standard deviation in spiked samples varied from 7 to 9.

Table 1
Recovery of diazepam from spiked cold drinks

Sample	Extraction with chloroform		Extraction with ether	
	Recovery (%)	R.S.D.	Recovery (%)	R.S.D.
Maza	84±8	9	74±5	7
Coke	84±6	7	78±7	9

Results are the average value of five observations (mean±S.D.).

Table 2
Recovery of diazepam from simulated cold drinks

Sample	Extraction with chloroform		Extraction with ether	
	Recovery (%)	R.S.D.	Recovery (%)	R.S.D.
Fanta	70±2	3	75±4	5
Thumps Up	95±8	8	84±5	6
Limca	70±2	3	69±1	2
Maza	79±3	3	86±7	8
Coke	78±7	9	84±5	9

Results are the average value of five observations (mean±S.D.).

The results of the simulation studies in chloroform extraction (Table 2) demonstrated considerably low recoveries of diazepam in citrus flavor-based cold drinks (Fanta and Limca). In ether extraction (Table 2) only Limca (69%) gave lower recovery as compared to Fanta (75%). The reason for low recoveries of diazepam in Fanta and Thumps up may be attributed to the interference of matrix of its food constituents. The relative standard deviation in simulated samples ranges from 3 to 9 in chloroform extraction and 2 to 9 in ether extraction.

The other organic solvents were also tried for extraction of diazepam but optimum results were obtained only in the chloroform and ether extracts with least emulsions formation possibilities and interference from food constituents.

The method described in this paper can be used for carrying out rapid and routine examination of diazepam in the carbonated and fruit drinks within acceptable limit of accuracy and precision by the law enforcement laboratories as the method is reliable, involve one-step extraction and less expertise is required in comparison to GC, HPLC and GC–MS.

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