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Determination of biogenic amines in beer with pre-column derivatization by high performance liquid chromatography

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ABSTRACT

Eighteen samples of commercially available Chinese beer were analyzed in order to determine the content of biogenic amines. The method involves pre-column derivatization of the amines with 4-chloro-3,5-dinitrobenzotrifluoride (CNBF) and subsequent analysis by RP-HPLC (reversed phase-high performance liquid chromatography) with diode array detection. The labeled biogenic amines were separated on a Kromasil C18 column (250 mm × 4.6 mm, 5 μm) at room temperature and UV detection was applied at 254 nm. The separation of seven labeled biogenic amines was achieved within 22 min by elution acetonitrile and HAc–NaAc buffers. The method linearity, calculated for each biogenic amine, has a correlation coefficient higher than 0.9925, in concentrations ranging from 2.9 μmol L^{-1} to 565 μmol L^{-1}. Detection limits of biogenic amines were 0.056–0.87 μmol L^{-1}, at a signal-to-noise ratio of 3. The proposed method has been applied to the quantitative determination of spermine, phenethylamine, spermidine, histamine, tyramine, tryptamine and putrescine in beer with recoveries of 91.9–103.1% and R.S.D. of 2.86–5.63%. Quantitation is relative to external standards. The results showed that each kind of beer examined contained at least three biogenic amines. Putrescine, histamine and tyramine were detected in all samples. Spermidine was detected in 89% of the beers. Spermine, tryptamine and phenylethylamine occurred in 78%, 61% and 44% of the beers examined, respectively. These levels were below the level that may elicit direct adverse reactions for most consumers.

1. Introduction

Biogenic amines are nitrogenous low molecular weight organic compounds which are derived mainly from amino acids through substrate-specific decarboxylase enzymes [1–4]. They are normal constituents of many foods and beverages and have been found to occur in cheese, wine, beer, fishery products and aged meat [5–12]. Biogenic amines are considered indicators of food quality and freshness since they are associated to the degree of food fermentation or degradation. For this reason, they contain a health risk for sensitive individuals. Their presence in high amounts in foods may induce several health disorders in sensitive humans such as nausea, respiratorial discomfort, hot flushes, cold sweat, palpitations, headaches, red rash, hyper/hypo tension, etc. [13–15]. In view of the possible harmful effects of biogenic amines, their concentration levels in foods deserve careful investigation.

Beer has been commonly reported, among foods and beverages, to be a health risk for some consumers due to the biogenic amines it contains [16,17]. The types and levels of biogenic amines in beers are affected mainly by raw materials, brewing techniques and hygienic conditions. Generally, histamine, tyramine, phenylethylamine and putrescine are usually found as the main biogenic amines in beer [18]. Histamine and tyramine are known to be the main cause of food intoxication, although other amines such as putrescine, cadaverine and phenylethylamine are also important since they may intensify the undesirable effect of histamine [19,20]. Furthermore, the interaction between ethanol and amines seems to be synergistic [21]. Due to the high consumption of beer and the possible harmful effects of biogenic amines, it is important to determine their levels.

Determination of biogenic amines is not simple because of their structure and usually present at low levels in a complex matrix. Biogenic amines are usually determined by separation techniques like thin layer chromatography (TLC) [22–24], capillary electrophoresis (CE) [17,25–29], gas chromatography (GC) [30–34] and High performance liquid chromatography (HPLC) [21,35–49]. HPLC is the technique most extensively used due to its high resolution, sensitivity, great versatility, and simple sample treatment. Biogenic amines do not exhibit satisfactory absorption at the visible or ultraviolet wavelengths, nor do they exhibit fluorescence. Therefore, many derivatization agents is usually applied for their analysis such as 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) [40], *N*-(2-acridonyl)-maleimide (MIAC) [48], benzoyl chloride [38], bis [2-ethylhexyl] sulphosuccinate (AOT) [41], dabsyl chloride (Dbs-Cl)

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Table 1
List of the analyzed beer samples.

Sample no.	Varieties	Beer-making areas (province)	Alcoholic content (v/v, %)	Original gravity (°P)	pH
1	Heineken	Shanghai	≥4.7	11.4	3.98
2	Carlsberg	Guangdong	≥4.0	10.3	4.03
3	Tiger	Shanghai	≥4.7	11.8	4.25
4	Corona extra	Wuhan	4.6	11.3	4.19
5	Yanjing	Beijing	≥3.6	10.0	4.13
6	Yanjing (alcohol free)	Beijing	≤0.5	–	4.34
7	Beijing	Beijing	≥4.0	11.0	4.27
8	Zhaori	Beijing	≥3.6	10.0	3.86
9	Haerbin	Haerbin	≥3.6	10.0	4.18
10	Tsingtao	Qingdao	≥4.3	11.0	4.22
11	Laoshan	Qingdao	≥4.0	10.0	4.31
12	Shanshui	Qingdao	≥3.6	10.0	4.12
13	Snow	Beijing	≥3.6	10.0	4.25
14	Budweiser	Wuhan	≥4.6	9.0	3.99
15	Landai	Beijing	≥4.0	11.0	4.17
16	Landai (black beer)	Beijing	≥4.5	13.0	4.15
17	Lanbei	Hebei	≥4.0	11.0	4.10
18	Lanjian	Hebei	≥3.6	9	4.36

[21,36,37], dansyl chloride (Dns-Cl) [43,44,46], 3,5-dinitrobenzoyl chloride (DNBZ-Cl) [35], 9-fluorenylmethyl chloroformate (FMOC) [51], 9-fluorenylmethyloxy carbonyl chloride (Fmoc-Cl) [45], *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (Fmoc-Osu) [39], *N*-hydroxysuccinimidyl fluorescein-o-acetate (SIFA) [49], 8-phenyl-(4-oxy-acetic acid *N*-hydroxysuccinimideester)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (TMPAB-OSu) [42], and o-phthalaldehyde (OPA) [47].

4-Chloro-3,5-dinitrobenzotrifluoride (CNBF) is an important fine chemical, which has been known to react with primary or secondary amines in presence of base to produce stable *N*-substituted-2,6-dinitro-4-(trifluoromethyl)-benzamine derivatives which are satisfactory ultraviolet absorption [50–54]. The levels of biogenic amines in American, Bulgarian, Brazilian and European beers have been reported [17,39,55,56]. However, no information was found on levels of biogenic amines in Chinese beers. In this work, a highly sensitive method to determine biogenic amines in beer samples by pre-column labeling with 4-Chloro-3,5-dinitrobenzotrifluoride is described.

2. Materials and methods

2.1. Instrumentation and conditions

A high performance liquid chromatography system, which consisted of two LC-10ATvp pumps and an SPD-10Avp, ultraviolet detector (Shimadzu, Japan) was used for the analysis and separation. A reversed-phase Kromasil ODS C18 column (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation at ambient temperature and Chromato Solution Light Chemstation for LC system was employed to acquire and process chromatographic data.

2.2. Chemicals and reagents

Seven biogenic amines (putrescine dihydrochloride, histamine dihydrochloride, tyramine hydrochloride, phenylethylamine, spermine, spermidine, tryptamine; ≥99.0%) were purchased from J&K Chemical Co. Ltd. (Beijing, China). An individual standard solution of 0.01 mol L^{-1} of each amine was prepared in water and further diluted to the required concentration when used. Working standards were prepared by mixing aliquots of the stock solutions and diluting with water. The stock and working standards were stored in dark at 4 °C when not in use. Acetonitrile and methanol were HPLC grade and purchased from J.T. Baker (USA). Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA). 4-Chloro-3,5-dinitrobenzotrifluoride was obtained from Alfa Aesar (Ward Hill, MA, USA) and its solution was prepared in methanol and filtered through a 0.45 μm nylon membrane filter and refrigerated when not in use. All other chemicals and solvents were analytical grade and from commercial sources. H_3BO_3–$Na_2B_4O_7$ buffer was prepared by mixing 0.2 mol L^{-1} H_3BO_3 solution with 0.05 mol L^{-1} $Na_2B_4O_7$ solution to the required pH value. The phosphate buffer was prepared by dissolving K_2HPO_4 in water, and then the pH was adjusted to the required value by adding concentrated H_3PO_4. HAc–NaAc buffer was prepared by mixing 0.1 mol L^{-1} HAc solution with 0.1 mol L^{-1} NaAc solution to the required pH value.

2.3. Chromatographic method

Before the analysis, the C18 column equipped with a guard column (4 mm × 3 mm i.d.) was pre-equilibrated with the mobile phase for 30 min. The HPLC separation of CNBF derivatives was carried out on Kromasil ODS C18 column by a gradient elution. Acetonitrile (eluent A) and HAc–NaAc buffer (10 mmol L^{-1}, pH 6.2) (eluent B) were used as mobile phase. All the solvents were filtered with a 0.45 μm membrane filter. The program was set for a liner gradient starting from 70% of solvent A to reach 100% of the solvent at 22 min the injection volume was 20 μL, and detection wavelength 254 nm. The flow rate was constant at 1.0 mL min^{-1} and the column temperature was at room temperature.

2.4. Derivatization procedure

To a 1.0 mL vial containing 200 μL of mixed biogenic amines solution (0.5 mmol L^{-1}), 300 μL of H_3BO_3–$Na_2B_4O_7$ buffer (pH 9.5), 100 μL of CNBF methanol solution (18.5 mmol L^{-1}) was added. After the whole solution was diluted to 1.0 mL with water, it was incubated at 60 °C for 30 min, and 2 M HCl (10 μL) were added to quench the reaction. The resulting solutions were filtered through 0.45 μm nylon filters and injected in the chromatographic system. Each sample was assayed in triplicate and all the assays were carried out at ambient temperature.

2.5. Analysis of beer samples

Eighteen samples of beer varieties from eight different manufacturers in four typical beer-making areas (Table 1) were purchased at local supermarkets. Beer were filtered through a 0.45 μm membrane filter and stored in glass bottles in a refrigerator. An aliquot of 1 mL of diluted samples or 1 mL of composite amine standards

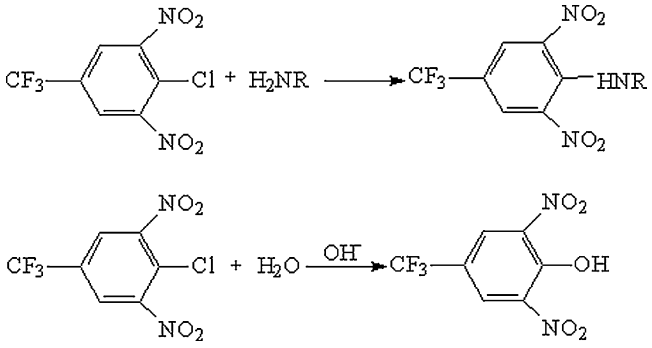

Fig. 1. The reaction scheme of CNBF with biogenic amines and water, respectively.

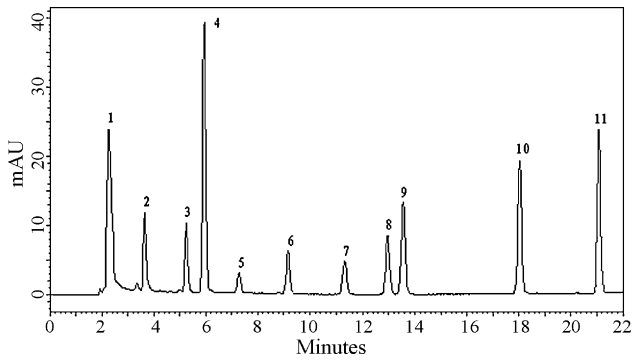

Fig. 2. HPLC chromatogram of the CNBF derivatives of biogenic amines standard solution. Peaks: 1: (CNBF)OH, 2: histamine, 3: unknown, 4: CNBF, 5: histamine, 6: tryptamine, 7: phenylethylamine, 8: tyramine, 9: putrescine, 10: spermidine, 11: spermine

was transferred to a vial, adjusted to pH 9.5 with reaction buffer and water added to 3 mL. After thorough mixing on a vortex-mixer, 2 mL of CNBF solution was added and it was mixed again. The mixture was heated in a water-bath for 30 min at 60 °C, shaking at 10 min and 20 min. The reaction was quenched by addition of 50 μL 2 M HCl. The resulting solutions were filtered through 0.45 μm nylon filters and injected in the chromatographic system. Quantitation is relative to external standards.

3. Results and discussion

3.1. Optimization of derivatization and HPLC conditions

The reaction of CNBF with amino groups on biogenic amines molecules is represented in Fig. 1. CNBF is known to have good activity and selectivity for amino compounds and to be employed as an excellent active group. They can react with amines in low concentration to form stable derivatives under base conditions, and the excessive reagents are hydrolyzed to corresponding phenol without any byproducts and interference. The hydrolysis compound can be written as (CNBF)OH. Because CNBF has relatively poor solubility in water, organic solvent should be added to the derivatization medium to avoid the precipitation of the reagent and derivatives. Compared acetonitrile with methanol, the first has higher mammalian toxicity than the later. So at least 100 μL of methanol should be added to the derivatization medium. There is a competition between the labeling and the hydrolysis, so excess labeling reagents should be used.

The influence of the amount of reagent on the derivatization was investigated. An aliquot of a mixture of the amines was reacted with various concentrations of CNBF (7.4×10^{-3} mol L^{-1}, 11.1×10^{-3} mol L^{-1}, 18.5×10^{-3} mol L^{-1} and 25.9×10^{-3} mol L^{-1}). The results shown that the peak areas of derivatives are highest and unchangeable when the concentration of reagent reached 18.5×10^{-3} mol L^{-1}, and there was not a statistically significant difference between 18.5×10^{-3} mol L^{-1} and 25.9×10^{-3} mol L^{-1}. The best proportion between biogenic amine and CNBF is 1:2.6. Although the excess of derivative reagent does not affect the results, it can lead to waste of CNBF. So, 18.5×10^{-3} mol L^{-1} was selected as the optimal concentration. The reaction of CNBF with amines was also found to be pH dependent. The influence of various pH values on the peak areas was also studied by using borate buffer. The optimum reaction pH was determined by derivatizing each of the seven amines at pH values ranging from 7.5 to 11.0. The results showed that the peak areas of the derivatives are steady at pH 8.5–10.0. This was probably due to deprotonation of amines at the alkaline conditions, which can promote the nucleophilic addition, as observed in the case of aliphatic diamines [57]. Hence, an optimum derivatization pH of 9.5 was selected for all subsequent experiments. Temperature is a very important factor in optimizing the derivatization rate. Therefore, the values ranging from 40 °C to 70 °C have been performed to find the best derivative temperature. It was found peak areas of the derivatives reached plateau at 60 °C. The reaction time is a critical factor for the derivatization reaction. The effect of reaction time on derivatization was studied over the period of 10–40 min, while keeping all the other parameters constant. It was clear that peak areas of all the derivatives of biogenic amines, excepting spermine, which required 40 min, reached an optimum value over a period of 20–30 min. In order to keep the total analysis time short, reaction time of 30 min was chosen.

The mobile phase composition was optimized in order to achieve fast and optimum separation of seven derivatives of biogenic amines. Chromatographic separations were carried out under gradient reversed-phase condition on Kromasil C18 column. Acetonitrile (eluent A) and HAc–NaAc buffer (10 mmol L^{-1}, pH 6.2) (eluent B) were used as mobile phase. The program was set for a liner gradient starting from 70% of solvent A to reach 100% of the solvent at 22 min, which gave the best separation within shortest analysis time. The effect of the buffers on the separation has also been investigated by using phosphate and acetate buffers. For high percentage of acetonitrile, the use of phosphate concentrations greater than 0.1 mol L^{-1} occasionally gave rise to precipitation problems (most likely disodium hydrogen phosphate), thus, acetate buffers was employed. The pH value of buffer in mobile phase was studied. In this experiment, the retention time of each derivative had no obvious change as the pH value varied from 4.0 to 7.0, which have been usually used in the mobile phase of HPLC, and the peak area of each derivative also indicated that CNBF derivatives were pH-insensitive and stable. In this experiment, pH 6.2 was used. The chromatogram of biogenic amines derivatives with CNBF obtained in gradient elution mode is shown in (Fig. 2). From Fig. 2, there was only one derivative for each biogenic amine (putrescine, tyramine, phenylethylamine, spermine, spermidine, tryptamine), excepting histamine had two derivatives at 3.6 min and 7.1 min. The separation of the derivatives was completed within 22 min.

3.2. Stability of the derivatives

The stability of biogenic amines derivatives with CNBF in methanol–water (1:9, v/v) at room temperature was investigated over 4 days without light irradiation. And no significant change in peak area of the derivatives was found. Derivatives in methanol–water (1:9, v/v) also show no significant change in the absolute peak area when exposed to ordinary light from a 100 W bulb for about 24 h. They are photostable. It appears that the derivatives of biogenic amines are very stable, as evidenced by the fact that the degradation rates of all the derivatives were less than 5% when analyzed by HPLC after 7 days of standing at room temperature.

Table 2
Linear calibration ranges, regression equations and detection limits of CNBF-amine derivatives.

Biogenic amines	Calibration range (μmol L^{-1})	Regression equation, *Y*	R^2	R.S.D. (%) *n* = 6 within-day	R.S.D. (%) *n* = 6 between-day	Detection limit (μmol L^{-1})[a]
Putrescine	11.3–565	128411*X* + 114650	0.9955	2.40	2.83	0.056
Histamine	9.0–450	57233*X* + 41235	0.9984	1.67	1.96	0.67
Tyramine	5.8–290	70927*X* + 29253	0.9958	3.12	3.22	0.58
Phenylethylamine	8.3–415	144500*X* − 152504	0.9980	1.48	2.30	0.20
Spermine	2.9–145	282063*X* − 804486	0.9946	2.58	2.86	0.87
Spermidine	6.9–345	146192*X* + 22618	0.9994	1.79	1.92	0.37
Tryptamine	6.2–310	12745*X* − 20912	0.9925	1.94	2.00	0.31

X: concentration of amine (μmol L^{-1}); *Y*: peak area of amine derivatives.
[a] *S*/*N* = 3, per 20 μL injection volume.

3.3. Method validation

A test mixture with different concentrations of standard biogenic amines was prepared and analyzed by using the optimized derivatization procedure and separation conditions for the determination of biogenic amines. The detection limits of biogenic amines were calculated as the amounts of biogenic amines that resulted in a peak three times higher than that of the baseline noise. The linear calibration ranges, regression equations, and detection limits of biogenic amines were calculated and the results were listed in Table 2. The correlation coefficients for these biogenic amines are from 0.9925 to 0.9994. The R.S.D. for the CNBF derivatives is from 1.48% to 3.12% for within-day determination (*n* = 6) and from 1.92% to 3.22% for between-day determination (*n* = 6). The detection limits for the labeled amines range from 0.05 μmol L^{-1} for putrescine to 0.87 μmol L^{-1} for spermine. It was shown that the quantification of biogenic amines could be well done with this method.

3.4. Application to sample analysis

The applicability of the proposed method was evaluated in beer samples. Biogenic amines were identified by adding standards to the samples. The results obtained from the analysis of samples were shown in Table 3. The data given were real contents of biogenic amines in samples through conversion. The chromatograms of samples and spiked standards were presented in Fig. 3. The recoveries of putrescine, histamine, tyramine, phenylethylamine, spermine, spermidine, tryptamine from different matrices were from 91.9% to 103.1% and R.S.D.s from 2.86% to 5.63%, depending on the sample investigated.

3.5. Amounts of BAs in beer

Our purpose was to determine the kinds of biogenic amines in beer samples from different producing areas and manufacturers made in China. Table 4 shows the results of the concentration of biogenic amines of beers from different manufacturers. The results illustrated that each kind of beer examined contained at least three biogenic amines. Putrescine, histamine and tyramine were detected in all samples. Spermidine was detected in 89% of the beers. Spermine, tryptamine and phenylethylamine occurred in 78%, 61% and 44% of the beers, respectively. In the beer samples studied, the levels of seven amines were 2.12–8.23 mg L^{-1} for putrescine, 2.96–7.15 mg L^{-1} for tyramine, 0.65–4.62 mg L^{-1} for histamine, 0–2.55 mg L^{-1} for spermidine, 0–3.96 mg L^{-1} for spermine, 0–1.73 mg L^{-1} for tryptamine and 0–0.42 mg L^{-1} for phenylethylamine. These values are similar or lower than those reported in the literature [17,39,55,56]. Fig. 4 represents the mainly biogenic amines in beer samples. The threshold levels for intoxication in humans by amines are very difficult to establish, because they depend on individual responses and the presence of other amines [58,59]. Ten Brink et al. [59] reported that 100–800 mg kg^{-1} of tyramine in foods are toxic; while Silla Santos [60] suggested that more than 1000 mg kg^{-1} (total amines in food) was dangerous for health.

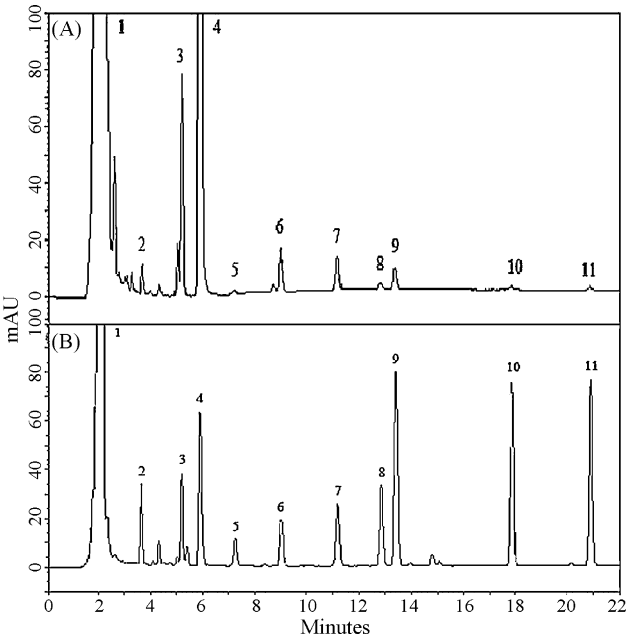

Fig. 3. Chromatograms obtained from samples (A) beer; (B) beer spiked with 250 μmol L^{-1} of standard biogenic amines. Peaks: 1: (CNBF)OH, 2: histamine, 3: unknown, 4: CNBF, 5: histamine, 6: tryptamine, 7: phenylethylamine, 8: tyramine, 9: putrescine, 10: spermidine, 11: spermine

Table 3
Analytical results of beer samples.

Biogenic amines[a]	Added (μg mL^{-1})	Found (μg mL^{-1})	R.S.D. (%, *n* = 6)	Recovery (%)
Putrescine	0, 68.0	5.7, 72.0	2.86	97.5
Histamine	0, 64.0	4.6, 69.2	3.39	100.9
Tyramine	0, 10.0	3.8, 10.4	4.87	100.4
Phenylethylamine	0, 70.0	1.4, 65.7	3.62	91.9
Spermine	0, 20.0	4.6, 19.0	5.63	92.7
Spermidine	0, 84.0	2.4, 89.0	4.29	103.1
Tryptamine	0, 94.0	3.8, 95.5	5.58	97.6

[a] Each biogenic amine was determinated in triplicate injections.

Table 4
Biogenic amines content of the analyzed Chinese beers.

Sample no.[a]	Concentrations of biogenic amines (μg mL^{-1}) (mean ± standard deviation)						
	HIST	TRYP	PHE	TYR	PUT	SPD	SPM
1	1.20 ± 0.04	ND[b]	0.12 ± 0.02	6.35 ± 0.06	4.25 ± 0.08	1.00 ± 0.04	2.64 ± 0.05
2	2.80 ± 0.16	0.45 ± 0.02	ND	5.08 ± 0.12	2.12 ± 0.14	ND	1.06 ± 0.02
3	0.96 ± 0.03	1.62 ± 0.05	ND	5.65 ± 0.03	2.90 ± 0.06	1.35 ± 0.03	ND
4	1.25 ± 0.03	0.36 ± 0.03	ND	3.47 ± 0.05	4.55 ± 0.05	0.71 ± 0.08	2.49 ± 0.08
5	4.62 ± 0.13	ND	ND	3.80 ± 0.03	5.73 ± 0.08	1.41 ± 0.14	3.96 ± 0.11
6	2.40 ± 0.02	ND	ND	4.30 ± 0.05	3.90 ± 0.05	1.65 ± 0.03	0.85 ± 0.03
7	0.83 ± 0.03	0.50 ± 0.03	0.42 ± 0.03	3.75 ± 0.08	7.06 ± 0.05	2.42 ± 0.08	2.62 ± 0.05
8	0.85 ± 0.02	1.22 ± 0.03	ND	6.10 ± 0.16	3.55 ± 0.20	1.76 ± 0.02	ND
9	1.05 ± 0.03	1.05 ± 0.05	0.10 ± 0.05	4.70 ± 0.02	3.79 ± 0.07	2.00 ± 0.02	0.94 ± 0.05
10	1.31 ± 0.03	1.45 ± 0.08	0.34 ± 0.08	2.96 ± 0.04	7.46 ± 0.08	1.86 ± 0.05	0.48 ± 0.02
11	1.08 ± 0.04	0.40 ± 0.04	ND	7.15 ± 0.05	5.12 ± 0.05	ND	0.69 ± 0.02
12	1.36 ± 0.03	1.73 ± 0.05	ND	4.35 ± 0.08	8.23 ± 0.16	1.40 ± 0.05	2.55 ± 0.03
13	2.34 ± 0.06	ND	0.26 ± 0.04	3.95 ± 0.03	4.29 ± 0.03	2.08 ± 0.02	ND
14	0.65 ± 0.03	ND	ND	7.04 ± 0.06	2.63 ± 0.02	2.36 ± 0.10	ND
15	1.78 ± 0.02	ND	0.20 ± 0.02	4.92 ± 0.05	6.14 ± 0.05	2.55 ± 0.02	1.68 ± 0.05
16	3.58 ± 0.10	ND	ND	2.90 ± 0.12	3.68 ± 0.08	1.20 ± 0.04	3.05 ± 0.04
17	0.69 ± 0.05	0.38 ± 0.02	0.42 ± 0.03	6.25 ± 0.03	4.05 ± 0.05	1.68 ± 0.04	2.46 ± 0.08
18	2.38 ± 0.05	0.64 ± 0.05	0.38 ± 0.02	4.84 ± 0.03	6.58 ± 0.03	1.70 ± 0.03	1.95 ± 0.05

[a] Each sample was determinated in triplicate injections.
[b] ND: not detected.

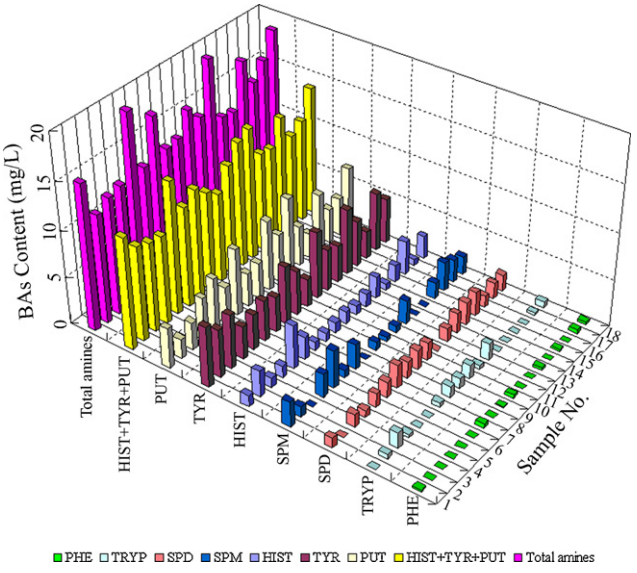

Fig. 4. Beers with mainly amine levels based on biogenic amine content.

The total biogenic amine levels of Chinese beers tested were lower than values considered as dangerous for health.

4. Conclusions

We have developed a new method for the determination of biogenic amines in beer and yielded satisfactory results. 4-Chloro-3,5-dinitrobenzotrifluoride, which has two NO_2 and one CF_3 that are all the strongest electron-withdrawing groups, is a compound that has not been reported previously as a derivatization reagent to detect biogenic amine. The derivatives of biogenic amines are stable under light irradiation and room temperature in methanol–water samples, which made the accuracy of quantitative biogenic amine available. The detection limits of biogenic amines are 0.056–0.87 μmol L^{-1}, at a signal-to-noise ratio of 3, which is equivalent or better than previously reported. The proposed method has been applied to the quantitative determination of spermine, phenethylamine, spermidine, histamine, tyramine, tryptamine and putrescine in beer with recoveries of 91.9–103.1%. Using the proposed method, we determined the contents of biogenic amines of 18 different beers produced by different manufacturers. The results showed that the beer samples contained at least three kinds of biogenic amines, and demonstrated that this is a useful, simple and rapid method for the separation, identification and determination of biogenic amines in many types of these samples. This work displays rational design strategy for the further investigations of novel and potentially useful ultraviolet analysis that can render better sensitivity as well as these advantages CNBF possesses.

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