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Discrepancies in determination of biogenic amines in beer samples by reversed phase and hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry

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Abstract

Biogenic amines (BAs) are nitrogenous organic bases occurring mainly in fermented food and beverages as a result of free amino acids bacterial decarboxylation. The reversed phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) based methods were compared in terms of usefulness for determination of BAs in beer samples. Analysis of BAs with the use of RPLC method were carried out after their derivatization with p-toluenesulfonyl chloride (tosyl chloride), while for HILIC one sample preparation consisted of only dilution. For RPLC method the limits of detection (LODs) and quantitation (LOQs) were in the range 0.54-4.3 ng/mL and 1.6–13 ng/mL, respectively. The obtained recoveries were from 75 to 125% with coefficient of variation (CV) less than 8%. The developed HILIC based method turned out to be less sensitive and not specific sufficiently for the determination of most BAs in native state in beer samples. In this case, the LODs and LOQs values were in the range 12-94 ng/mL and 35–290 ng/mL, respectively.The observed matrix effects during analysis of beer samples were significant enough to distort the BAs content. The obtained recoveries were often below 75% with CVs less than 11%. Finally, both developed methods were applied for analysis of BAs in samples of lager beers. Due to the low recoveries and strong influence of matrix the HILIC method could be only applied to qualitative analysis of some BAs in beer samples. Regardless of the relatively lengthy sample preparation for RPLC method (time of derivatization – 2h), it was proven that a derivatization reaction is required for such matrix as beer.

Keywords: biogenic amines; hydrophilic interaction liquid chromatography; reversed phase liquid chromatography; mass spectrometry; derivatization

1. Introduction

Biogenic amines (BAs) are nitrogenous organic bases, occurring mainly in fermented food and beverages as a result of bacterial decarboxylation of free amino acids. The decarboxylase enzyme transforms amino acid to BA by removal of its carboxyl group. BAs could be formed also by amination and transamination of aldehydes and ketones. In non-fermented foods, BAs may be present as a result of undesirable microbial activity but at lower concentrations than those from controlled fermentation [1–3].

The BAs are present practically in most processed and stored food products, such as meat, fish, cheese, sauerkraut and beverages such as wine or beer [1,3,4]. In most cases, the concentration of BAs is at trace levels, however in some cases the content of BAs is significant. In numerous cases, the BAs reach concentration at the level mg/kg, for example: histamine in fish - 10000 mg/kg or tyramine in ripened cheese - 2520 mg/kg, where the norm set by European Community [5] are 100 mg/kg for histamine [4]. In case of tyramine, the maximum allowable level in food and beverages is 100-800 mg/kg [1]. Despite this, there is no legislation that provides acceptable norms in food and beverages for the other BAs. There is only one record published by the European Food Safety Authority (EFSA) [6], presenting the content of BAs may increase in fermented products during storage and it should be monitored [1,4,6–8].

High content of BAs in food and beverages can be toxic to humans, due to the fact that high content of these compounds causes disturbances in the organism, because of their strong physiological activity. It is worth noting that some aromatic amines, for example tyramine or tryptamine cause vasoconstrictor action while histamine and others lead to vasodilation [1,9]. Moreover, high levels of some BAs in food, such as histamine may cause nausea, diarrhea, rash, hypotension and headache while tyramine – hypertension and migraine [4,9]. Additionally, presence of other BAs (mainly putrescine and cadaverine) increases synergic toxicity of histamine and tyramine because of inhibition of intestinal enzymes that metabolize them[6,9]. Furthermore, some of them, mainly polyamines (cadaverine, putrescine, spermine and spermidine) may be responsible for the formation of carcinogenic nitrosamines through their reaction with nitrites [1,6]. The high content of BAs in beverages (wine or beer) may be problematic because ethanol can inhibit activity of monoamine oxidase (MAO) and diamine oxidase (DAO), thereby delaying decomposition of these compounds in the organism. Additionally, acetaldehyde and anti-depressive drugs cause the same interferences [10]. Therefore it is important to control the level of BAs in food and beverages [11].

To determine BAs in food and beverage samples many analytical techniques are used. The most popular techniques are: gas chromatography (GC), capillary electrophoresis (CE), thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) [1], whereby the last one seems to be the most common [11,12]. In reference to the literature, many methods based on reversed-phase liquid chromatography(RPLC) coupled with mass spectrometry (MS) or UV-Vis detection were used to determine BAs in samples of food and beverages [3,7,11,13–18]. The determination of BAs by RPLC coupled with MS in their native state is problematic, due to the high polarity of these compounds. Therefore, a very important step is sample preparation in order to eliminate problems such as low sensitivity or tailing peaks – generally derivatization is applied. Additionally, the use of derivatization reaction improves the resolution in reversed-phase mode [2,8,19]. On the other hand, derivatization step is time consuming and due to the implementation of additional step, recoveries may not be satisfactory [20].The choice of derivatizing agent depends on detector, chromatographic technique and structure of analysed compounds [2]. According to the literature, the most frequently chosen derivatizing agents in case of determination of primary and secondary BAs are dabsyl chloride (DBS-Cl) benzoyl chloride, 4-chloro-3,5-dinitrobenzotrifluoride (CNBF), o-phthalaldehyde (OPA), diethyl ethoxymethylenemalonate (DEEMM), 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), 9-H-fluoren-9-ylmethyl chloroformate (FMOC-Cl) or 1-fluoro-2,4-dinitrobenzene (DNBF) [2,21]. The commonly used derivatizing agents for the determination of polyamines compounds are dansyl chloride (DNS-Cl), tosyl chloride and benzoyl chloride [2,21]. One of the alternative approaches is to utilize the hydrophilic interaction liquid chromatography (HILIC) technique, which allows the determination of BAs in native states, thus time of sample preparation in this case is shorter [22,23]. In addition, HILIC mode of separation utilizes higher content of organic solvent in mobile the phase, which should favor ionization in mass spectrometry [23,24]. To our very best knowledge, few applications of the HILIC technique combined with MS for determination of underivatized BAs in food and beverage samples were published [23,25–27], but none of them described determination of these analytes in beer samples.
The aims of this research were to compare and find similarities/differences in terms of chromatographic parameters, linearity, recovery and separation of analytes for HILIC and RPLC techniques coupled with tandem mass spectrometry (MS/MS) for the determination of BAs in beer samples.

2. Material and Methods

2.1. Chemicals

Seventeen BAs: propylamine hydrochloride 99% (purity) (PA), dimethylamine hydrochloride 99% (DMA), ethylamine hydrochloride 98%(EA), diethylamine hydrochloride 99% (DEA), methylamine hydrochloride 99% (MA), tryptamine hydrochloride 99% (TRP), cadaverine dihydrochloride 98% (CAD), spermine tetrahydrochloride 99.5%(SPM), 2-phenylethylamine hydrochloride 98% (PHA), tyramine hydrochloride 98%(TYR), putrescine dihydrochloride 98% (PUT), histamine dihydrochloride 99% (HIS), butylamine 99% (BA), hexylamine 99%(HEA), isopentylamine 99% (isoPA), spermidine trihydrochloride 99.5%(SPD), agmatine sulfate 98% (AGM) and the internal standard (IS) – 1,7-diamoheptane 98% (DAH) – were acquired from Sigma Aldrich (St. Louis, USA). Boric acid and sodium hydroxide were purchased from POCH (Gliwice, Poland). Ammonium formate, acetonitrile (ACN) with LC–MS grade, and tosyl chloride (≥99%) were obtained from Sigma Aldrich (St. Louis, USA). Formic acid (FA) was purchased from Merck (Darmstadt, Germany). Nylon Captiva Econofilters (25 mm diameter, 0.2 µm pore size) were purchased from Agilent Technologies (Santa Clara, USA). Ultrapure water was prepared using the HLP5 system from Hydrolab (Wiślina, Poland).

2.2. Beer sample preparation

Six beer samples (B1-B6) differing in alcohol content (B1-4.0%, B2-5.2%, B3- 5.6%, B4 - 5.2%, B5 - 0.0%, B6 - 5.5%), type (B1-B4, B6 - lager, B5- non alcoholic lager) and place of production were purchased at the local supermarket. All beers were analyzed within one day from purchase.

For both methods (HILIC and RPLC), the beer samples were degassed in an ultrasonic bath for 10 minutes and diluted (1+9, v/v) with water (RPLC) or with mixture of ACN:0.1M HCl (3+7, v/v) (HILIC).

For RPLC method, procedure of derivatization with tosyl chloride was applied [11]. The choice of tosyl chloride as a derivatizing agent was dictated by the fact that the derivatization reaction products are more stable than when other agents are used. Additionally, tosyl chloride enables the derivatization of polyamines, which was desirable in the conducted research [28].

In order to carry out derivatization reaction, 500 µL of diluted beer was transferred to 15 mL plastic centrifuge tubes and mixed with 500 µL solution of tosyl chloride (10 mg/mL in

acetonitrile) and 250 µL borate buffer (0.5 M, pH=11). Borate buffer was prepared by titrating 0.5 M boric acid solution with sodium hydroxide to the required pH value. Next, the solutions were shaken in thermomixer (Eppendorf, Hamburg, Germany) for 120 minutes at 50ºC. In the end, the samples were filtered through a 0.2 µm nylon filter.

In case of HILIC method, sample preparation consisted of only two steps: dilution and filtration through a 0.2 µm nylon filter.

Finally, prepared samples were injected into the chromatographic system. The sample preparation procedure for both methods is shown in Fig. **1**.

<insert Figure 1>

2.3. Preparation of standard solutions

Individual stock solutions (500 µg/mL) of BAs and IS were prepared in 0.1M HCl to maintain their stability. To prepare the standard mix, 1 mL of each of seventeen solutions were introduced into the 25 mL volumetric flask and filled up to the mark with ACN:0.1 M HCl (3+7, v/v) mixture. The concentration of each BA in standard mix was 20 µg/mL. Prepared standard mix was used to construct the calibration curves for RPLC and HILIC methods.

2.4. Instrumentation

All analyses were performed by Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Japan) equipped with the ESI source. In case of both methods (RPLC and HILIC) the ESI source was operated in positive ion mode with the following conditions: the source temperature was set at 300˚C, ion spray voltage was set at 4kV and nebulizing gas flow, heating gas flow and drying gas flow were set at 3, 10 and 10 L/min respectively. Temperature of the desolvation line and heat block were set as follows: 250˚C and 400˚C. Each BA was monitored by its two most intense MRM transitions. Conditions of ion transitions were chosen separately for the HILIC mode and RPLC mode. Source and MS parameters for both methods (RPLC and HILIC) are shown in **Table S1** (Supplementary material). Data acquisition and analysis were accomplished with LabSolutions 5.60 SP1 software. The chromatographic separation was done using the UPLC Nexera X2 System (Shimadzu) equipped with binary pump LC-30AD, degasser DGU-20A5R, controller CBM-20A, autosampler SIL-30AC and thermostated column oven CTO-20AC.

For the RPLC separation of the derivatized BAs, Kinetex C8 (100 x 2.1 mm, 1.7µm, Phenomenex) column was used. In case of HILIC method, Ascentis Express OH5 (150 x 2.1

mm, 2.7µm, Supelco) column was applied. Separation conditions for both methods are presented in **Table 1.**

<insert Table 1>

2.5. Calibration curves

For both methods, a set of eleven calibration solutions were prepared by mixing and diluting variable aliquots of the standard mix with mixture of ACN:0.1M HCl (3+7, v/v) to obtain 1, 5, 25, 50, 100, 250, 500, 750, 1000, 1250, 1500 and 1750 ng/mL of each analyte. Among eleven calibration solutions, a six – point range was selected individually for each BA to maintain the linearity of constructed calibration curves.

For RPLC method 100 µL of each calibration solution was mixed with 400 µL of ultrapure water and subjected to the derivatization procedure. In case of the HILIC method, calibration solutions of BA were injected directly. In all calibration solutions the concentration of IS (DAH) was kept at 50 ng/mL.

2.6. Matrix effects

For estimation of matrix influence fortification procedures were carried out for both developed methods. Suppression or enhancement of MS signal was checked by the standard addition method. For this purpose, a mix of BAs with appropriate concentrations (100, 150 and 200 ng/mL) was added to the beer samples. Fortified samples were diluted (1+9, v/v) with mixture of ACN:0.1M HCl (3+7, v/v) and in case of RPLC method subjected to derivatization reaction.

3. Results and discussion

3.1. Separation of analytes and mass spectrometry response; comparison of chromatographic conditions

The optimization of the methods was carried out with the use of a standard mix consisting of eighteen BAs including IS. The concentration of each BA in standard mix was 200 ng/mL. For RPLC method, standard mix was firstly subjected to the derivatization procedure.

3.1.1. RPLC based method

To develop RPLC method, the conditions described in the previous publication [11], were partially taken (mainly: temperature of column - 40ºC and composition of mobile phase – ACN and water acidified with FA 0.1% v/v). Other conditions such as flow rate, injection volume and gradient elution were optimized for the chosen column. It was observed that use of column Kinetex C8 (100 x 2.1 mm, 1.7µm) gave better results, in terms of peak shape and sensitivity than in case of column used in previous study (Gemini C-18, 150 x 4.6 mm, 3µm). Additionally, time of analysis has been reduced from 28 minutes to 15 minutes including equilibration time. Example chromatograms obtained with Kinetex C8 (RPLC method) column under optimized conditions are presented in **Fig. 2.**

<insert Figure 2>

3.1.2 HILIC based method

In case of HILIC method, five columns were tested: Ascentis Express Si, bioZen Glycan, ZIC®-HILIC, Kinetex Si, and Ascentis Express OH5. Different compositions of mobile phase (component A: ammonium acetate or ammonium acetate +0.1% v/v FA and ammonium formate or ammonium formate+0.1% v/v FA, component B: ACN or ACN+0.1% v/v FA) and different concentration of buffer solutions (20, 40, 80, 100, 150 mM) were investigated. It was observed that higher concentration of buffer solution (150mM) resulted in better separation of analytes and in reduction of peak tailing. It was found that addition of ammonium formate instead of ammonium acetate leads to improvement of peak shape. In case of ammonium acetate addition- the severe peak tailing and strong signal suppression were observed. It was observed that the content of FA up to 0.1% v/v in both components of mobile phase improves ionization. Results obtained with the use of five columns were compared in terms of analysis time, separation of analytes, peak shapes and intensity. The most problematic compound during separation optimization was SPD due to the severe peak tailing. With the use of Ascentis Express OH5 column the value of the tailing factor was significantly lowered in comparison to those obtained with use of the other columns. Based on the obtained results, it was decided to select this column for further analysis.

It should be emphasized, for selected column, although it gave the best results among all the tested ones, still noticeable distortion of peak shapes were observed. What is more, intensity of peaks – especially in situation, when BAs have low molecular mass, such as: MA, EA, PA and BA is not satisfactory, probably because of poor ionization. This is due to the fact, that fragmentation of these BAs is difficult to achieve - for example: low pseudomolecular ion

mass (32.1) of MA resulted in no observable fragment ions. Therefore, for this compound the pseudotransition was chosen (32.1→32.1) during flow injection analysis (FIA). However, the selected pseudotransition of MA in fact resulted in no observable peak even in the mixture, where its content was up to 1750 ng/ml. For EA two transitions (46→46, 46→27) were chosen, one of them was pseudotransition due to the lack of other specific fragments. In case of SPM, a problem with the determination of pseudomolecular ion and in consequence fragment ions appeared already at the MS optimization stage. Despite many attempts (changing mobile phases and MS parameters), it was not possible to determine the pseudomolecular ion or adduct ions which could be characteristic for this compound. In case of SPD determination regardless of the used conditions during optimization (columns, program of gradient elutions, composition of mobile phase), it was not possible to obtain narrow peak. Peak was tailing strongly, which gave non-linear response. Therefore, construction of linear calibration curve for this compound was problematic.

It is necessary to underline that the optimization of the HILIC based method was laborious and difficult since BAs differ significantly in their chemical structure (aliphatic, alicyclic and heterocyclic compounds).

Example chromatograms obtained with Ascentis Express OH5 (HILIC method) column under optimized conditions are presented in **Fig. 3.**

<insert Figure 3>

3.1.3. Comparison of RPLC and HILIC based methods

For the RPLC method analysis time is significantly shorter (15 minutes) compared to HILIC one, which should be expected due to the quicker mass transfer (smaller diameter of stationary phase particles). In addition, peaks were more symmetrical – peaks obtained for all analytes are tall and narrow. In case of the HILIC method, analysis time is longer (25 minutes) since the equilibration time of HILIC column is longer compared to the column used by the RPLC method.

Finally, two chromatographic methods (RPLC and HILIC) were developed which resulted in the possibility to separate seventeen and fourteen BAs respectively. For the HILIC based method, three of the seventeen BAs were not determinable.

3.2. Calibration

For both methods a six-point (each point in triplicate) calibration curve was constructed by plotting the ratio of the peak area of the analyte to the peak area of IS (DAH) versus

concentration. Limit of detection (LOD) values were estimated using the following formula: $LOD = 3.3 \times S_b/a$, where S_b is the standard deviation of intercept and a is the slope of the calibration curve. To estimate the limit of quantitation (LOQ) values the LOD values were multiplied by three.

-For RPLC method, two concentration ranges were used (1-250 ng/mL for sixteen BAs and 5-250 ng/mL for DMA).

In order to maintain linearity of calibration curves for HILIC method, different concentration ranges (25-1500 ng/mL for TYR, HIS, isoPA, FEA, TRP and DMA,50-1500 ng/mL for PA, AGM and DEA, 50-1750 ng/mL for HEA, 25-1750 ng/ mL for CAD, 25-1250 ng/mL for PUT and 100-1750 ng/mL for EA) for individual BAs were required (see Section 2.5). Furthermore, construction of calibration curves for compounds such as: MA, SPD and SPM was not possible. For MA and SPM problems appeared already at the optimization stage – for MA no peak was observed, while for SPM determination of pseudomolecular ions and fragment ions was not possible. For SPD non-linear response from MS detector was observed.

In other cases, obtained calibration curves were linear in the tested concentration ranges and were characterized by coefficients of determination higher than 0.99. The parameters of calibration curves for the HILIC and RPLC methods are presented in Table 2.

<insert Table 2>

RPLC method is characterized by lower LOD and LOQ values (respectively: 0.54-4.3 ng/mL, 1.6-13 ng/mL) than HILIC method (respectively: 12-94 ng/mL, 35–290 ng/mL). Generally, the slopes of the calibration curves for the RPLC method are greater than for the HILIC method, and consequently the LOD values are lower. Furthermore, the intercept of calibration curves for HILIC method indicates signal suppression for most BAs, except for AGM and HIS where there is a noticeable enhancement.

3.3. Matrix effects

Fortification procedures were carried out to estimate matrix effects. The procedure for the preparation of spiked samples was presented in **Section 2.6.**

3.3.1. Analysis of real samples by RPLC and HILIC methods

Real samples of beers were analyzed by RPLC and HILIC methods. For this purpose, six samples of beer differing in alcohol content, type and place of production were subjected to appropriate sample preparation according to the method used. The content of BAs in analyzed samples of beer and significance levels (P-values) are presented in **Table 3**.

<insert Table 3>

Obtained results in most cases differ significantly which was proven by calculating the difference between the observed means in two independent sample sets with 95% confidence interval. For the RPLC method the content of TRP in sample beer B1 is 144.54±0.72 ng/mL, while for HILIC method, the content is ten times lower (13.81±0.78 ng/mL). A similar situation is observed for CAD – in the RPLC method the content of this compound in sample B4 is 612.2±4.5 ng/mL and for HILIC method is 74.1±7.8 ng/mL. In addition, for the RPLC method, it was possible to determine more BAs than by HILIC method. Using the RPLC method, it was possible to determine fifteen BAs (PA and HEA were not detected in any of the analyzed samples). The HILIC method allowed the determination of only five BAs (AGM, PUT, TRP, CAD and TYR). The content of some BAs for the HILIC method were not possible to determine because of high values of LOD.

3.3.3. The standard addition method (comparison of results obtained by RPLC and HILIC methods)

Regarding Section **3.3.1**(differences in the content of BAs obtained by two methods) – the standard addition method was applied for both approaches (the conditions are described in **Section 2.6**). The main objective of this was to check the reliability of the obtained results. The recoveries of individual analytes are summarized in **Table 4.**

<insert Table 4>

The research proved that the matrix did not significantly affect the results obtained by the RPLC method. Recoveries for the RPLC method vary from 75 to 125% with CVs in the range of 0.06–7%, while for HILIC method, these values were often below 75% with CVs in the range of 0.03–11% (**Table 3 and Table 4**). For example, recoveries for CAD in samples B1 and B2 were respectively: 50% and 24%, which proves the strong suppression of the signal. For TRP in most samples (B1-B4, B6), recoveries were below 67%. In some beer samples, the content of BAs determined by the RPLC and HILIC methods seemed to be similar (for example: content of AGM in samples B1, B2, B4-B6 or content of PUT in B1-B6). However, according to the statistical test results for AGM, PUT, TRP, CAD and TYR in most cases differ significantly (P<0.05). For the HILIC method the~~However, according to the statistical test results for AGM, PUT, TRP, CAD and TYR in most cases differ significantly (P<0.05). For the HILIC method the~~ results are not reliable, probably due to the strong influence of the matrix. Beer sample is a complicated matrix, hence determining some BAs in native state turned out to be problematic, even with the HILIC method, which enables the determination of polar compounds. However, HILIC method can be used to determine BAs qualitatively, to prove their presence in beer samples. In our opinion HILIC based method should not be recommended for quality control or quantification of BAs in beer samples. Due to the fast sample preparation and relatively simple chromatographic conditions one can use the presented method for quick screening of presence of some BAs, especially those with more complex structure and higher mass >100Da. Examples of chromatograms obtained with RPLC method and HILIC method after analysis of beer sample (B3) are presented in **Fig. 4.**

<insert Figure 4>

Compared to the results of the research published in the previous publication, where the RPLC method was used for the determination of BAs in samples of wines and beers [11] - the content of most BAs in beer samples for methods based on RPLC technique is at the same concentrations level. For example, concentration of: AGM and TRP in the same type of beer samples are respectively 5981.8±6.6, 182.32±0.21 and previous -5831±186 and 163.0±5.4 ng/mL. In addition, HEA and PA were not detected in any of the beer samples.

4. Conclusions

Two methods for determination of BAs in beer samples based on HILIC and RPLC techniques coupled with tandem mass spectrometry (MS/MS) were compared in terms of

chromatographic parameters, linearity, recovery and separation. Using RPLC method it is possible to determine seventeen BAs while fifteen of them (PA and HEA were not detected) were identified in the beer samples. The HILIC based method allows to determine fourteen BAs, but in beer samples only five of them(AGM, PUT, TRP, CAD and TYR) were quantifiable. For both methods the prepared calibration curves were linear within the studied concentration ranges and were characterized by R^2 higher than 0.99. In case of HILIC method the LOD values obtained for all analytes are significantly higher (12-94 ng/mL) than in case of RPLC method (0.54-4.3 ng/mL). Furthermore, the sample fortification procedure showed, unlike the HILIC method, the RPLC one is characterized by higher precision and accuracy. Recoveries for RPLC method vary from 75 to 125% with CVs in the range of 0.1–7%, while in for HILIC method, recoveries were often below 75% with CVs less than 11%. ~~Only in case of PUT and AGM, the results obtained with the HILIC method were consistent with those obtained with the RPLC method. On the other hand, determination of BAs by RPLC based method is time-consuming mostly to the derivatization reaction.~~

The carried out research showed that the RPLC method enables the determination of seventeen BAs after derivatization with better accuracy and precision. In case of HILIC method, a strong influence of the matrix on the obtained results is observed, which means that this method is not suitable for the quantification of most BAs in the native states in beer samples. Despite the relatively lengthy sample preparation for RPLC method (about 2.5h), it was proven that derivatization reaction is required for such a complicated matrix to obtain reliable results.

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Table 3. Content of BAs [mean ± SD, [ng/mL] in analyzed samples of beer by RPLC and HILIC method and P-values.

Table 4. Recoveries of analytes in six beer samples [%].

Analyte	Calibration curve equation (6 points, n=3)	Range [ng/mL]	S_a	S_b	LOD [ng/mL]	LOQ [ng/mL]	R^2
MA	-	-	-	-	-	-	-
EA	y=0.0004x-0.003	100-1750	0.000011	0.012	94	290	0.997
DMA	y=0.0107x-0.175	25-1500	0.000081	0.064	20	60	0.9997
PA	y=0.0012x-0.047	50-1500	0.000014	0.012	33	100	0.9994
AGM	y=1.164x+23	50-1500	0.014	12	33	100	0.9995
BA	y=0.0042x-0.147	25-1500	0.000039	0.031	24	72	0.9996
DEA	y=0.0151x-0.62	50-1500	0.00019	0.16	35	110	0.9994
PUT	y=0.0213x-0.03	25-1250	0.00018	0.11	17	52	0.9997
TRP	y=0.0928x-2.01	25-1500	0.00083	0.66	24	71	0.9996
FEA	y=0.0612x-2.23	25-1500	0.00057	0.46	24	73	0.9996
isoPA	Y=0.005x-0.137	25-1500	0.000039	0.031	20	61	0.9997
HIS	y=0.081x+0.26	25-1500	0.00052	0.42	17	51	0.9998
CAD	y=0.032x-0.19	25-1750	0.00013	0.11	12	35	0.9999
HEA	y=0.0072x-0.17	50-1750	0.000097	0.10	46	140	0.9991
SPD	-	-	-	-	-	-	-
TYR	y=0.0246x-0.66	25-1500	0.00021	0.16	22	65	0.9997
SPM	-	-	-	-	-	-	-

Table S1. The parameters of ion transitions and conditions of the ESI source for a HILIC and RPLC methods

Table 2.

Quantification and validation data for biogenic amines for the HILIC (a) and RPLC (b) methods.

a.)

b.)

Analyte	Calibration curve equation (6 points, n=3)	Range [ng/mL]	S_a	S_b	LOD [ng/mL]	LOQ [ng/mL]	R^2
MA	y = 0.0004087x+0.016837	1-250	0.0000032	0.000064	0.60	1.8	0.998
EA	y = 0.006048x+0.0611	1-250	0.000086	0.0016	0.86	2.6	0.998
DMA	y = 0.01665x+0.461	5-250	0.00055	0.022	4.3	13	0.996
PA	y = 0.003124x+0.08363	1-250	0.000042	0.00078	0.82	2.5	0.998
AGM	y = 0.03227x+0.1905	1-250	0.00046	0.0085	0.87	2.6	0.9993
BA	y = 0.003652x+0.02751	1-250	0.000052	0.00096	0.87	2.6	0.9997
DEA	y = 0.01199x+0.1050	1-250	0.00019	0.0036	0.98	2.9	0.9997
PUT	y = 0.01344x+0.0995	1-250	0.00017	0.0031	0.76	2.3	0.9992
TRP	y = 0.005007x+0.0187	1-250	0.000077	0.0014	0.93	2.8	0.9993
FEA	y = 0.004882x+0.0277	1-250	0.000059	0.0011	0.73	2.2	0.9990
isoPA	y = 0.002015x+0.01855	1-250	0.000026	0.00048	0.78	2.4	0.9995
HIS	y = 0.1332x+0.622	1-250	0.0017	0.032	0.79	2.4	0.998
CAD	y = 0.007726x+0.0462	1-250	0.000069	0.0013	0.54	1.6	0.9997
HEA	y = 0.001803x+0.01090	1-250	0.000030	0.00054	0.99	3.0	0.9993
SPD	y = 0.01508x+0.1400	1-250	0.00024	0.0043	0.95	2.9	0.998
TYR	y = 0.006687x+0.0361	1-250	0.000077	0.0014	0.69	2.1	0.9998
SPM	y = 0.003951x+0.03966	1-250	0.000051	0.00094	0.79	2.4	0.998

***S_a**-standard deviation of the slope, **S_b**-standard deviation of the intercept, **R^2**- correlation coefficient, **LOD-** limit of detection, **LOQ-** limit of quantitation, **n-** number of measurements*

Table 1.

Separation conditions of the chromatographic system for the RPLC and HILIC methods.

	RPLC method	**HILIC method**
Column	Kinetex C8 (100 x 2.1 mm, 1.7µm)	Ascentis Express OH5 (150 x 2.1 mm, 2.7µm)
Flow rate [mL/min]	0.6	0.8
Temperature of column[°C]	40	22
Injection volume [µL]	1	2
Analysis time [min]	15	25
Mobile phase	A: H_2O 0.1% v/v FA B: ACN 0.1% v/v FA	A: 150 mM NH_4FA 0.1% v/v FA B: ACN 0.1% v/v FA
Gradient elution	0 →5 min 25-40%B 5→10 min 40-80%B 10→15 min 25%B	0 →10 min 98-70%B 10→18 min 70-20%B 18→25 min 95%B

Table 3.

Content of BAs [mean ± SD, [ng/mL] in analyzed samples of beer by RPLC and HILIC method and P-values.

BAs	**Method**	**B1**	**CV [%]**	**P-value**	**B2**	**CV [%]**	**P-value**	**B3**	**CV [%]**	**P-value**
AGM	HILIC RP	3660±290 3696±28	8 0.8	**0.84**	8420±150 8235±99	2 1	**0.15**	2740±140 3865±24	5 0.6	0.0002
PUT	HILIC RP	1563±20 1508.1±2.4	1 0.2	0.0092	3157±84 3202±44	3 1	**0.46**	3510±130 3301±53	4 2	**0.061**
HIS	HILIC RP	N/A N/A	- -	-	N/A 65.3±3.8	- 6	-	N/A 15.09±0.25	- 2	-
TRP	HILIC RP	13.81±0.78 144.54±0.72	6 0.5	0.0001	85.3±7.0 189.0±1.5	8 0.8	0.0001	46.1±2.3 162.42±0.17	5 0.1	0.0001
CAD	HILIC RP	164.10±0.63 277.8±5.7	0.4 2	0.0001	100±10 367.8±9.1	10 3	0.0001	290±14 278.86±0.16	5 0.1	**0.24**
TYR	HILIC RP	535±36 647±16	7 3	0.0079	253±21 426±12	8 3	0.0002	7490±150 6310±130	2 2	0.0005
MA	HILIC RP	N/A 992±54	- 5	-	N/A 1157±16	- 1	-	N/A 1200±56	- 5	-
EA	HILIC RP	N/A 94.9±1.2	- 1	-	N/A 105.1±4.6	- 4	-	N/A 122.42±0.67	- 1	-
DMA	HILIC RP	N/A 138.7±2.6	- 2	-	N/A 383.2±4.8	- 1	-	N/A 115.6±2.9	- 2	-
PA	HILIC RP	N/A N/A	- -	-	N/A N/A	- -	-	N/A N/A	- -	-

BA	HILIC RP	N/A 29.8±2.2	- 7	-	N/A 28.9±1.1	- 4	-	N/A 25.1±2.2	- 9	-
DEA	HILIC RP	N/A 17.07±0.81	- 5	-	N/A 18.32±0.79	- 4	-	N/A 10.48±0.15	- 1	-
HEA	HILIC RP	N/A N/A	- -	-	N/A N/A	- -	-	N/A N/A	- -	-
FEA	HILIC RP	N/A 34.6±1.7	- 5	-	N/A 33.3±1.2	- 4	-	N/A 37.3±1.1	- 3	-
IZOPA	HILIC RP	N/A 37.6±2.4	- 6	-	N/A 46.79±0.48	- 1	-	N/A 52.8±1.5	- 3	-
SPD	HILIC RP	N/A 41.009±0.031	- 0.1	-	N/A 913.8±9.1	- 1	-	N/A 1103.1±4.3	- 0.4	-
SPM	HILIC RP	N/A 309.1±5.6	- 2	-	N/A 571.0±9.6	- 2	-	N/A 247.1±4.3	- 2	-

BAs	**Method**	**B4**	**CV [%]**	**P-value**	**B5**	**CV [%]**	**P-value**	**B6**	**CV [%]**	**P-value**
AGM	HILIC RP	5740±210 5981.8±6.6	4 0.1	**0.12**	4712±35 4120±75	1 2	0.0002	8500±420 8000±110	5 1	**0.12**
PUT	HILIC RP	3550±81 3361.4±4.2	2 0.1	0.016	1964±30 1622.9±2.0	1 0.1	0.0001	3371.9±8.3 3258±30	0.3 0.9	0.003 2
HIS	HILIC RP	N/A 74.2±2.2	- 3	-	N/A 21.11±0.61	- 3	-	N/A 820.23±2.2	- 3	-
TRP	HILIC RP	61.16±0.51 182.32±0.21	1 0.1	0.0001	51.057±0.014 165.75±0.65	0.03 0.4	0.0001	88.1±7.1 180.66±0.47	8 0.3	0.000 1
CAD	HILIC RP	74.1±7.8 612.2±4.5	11 0.7	0.0001	142.7±6.4 284.6±1.8	4 0.6	0.0001	130.7±1.9 393.66±0.74	1 0.2	0.000 1
TYR	HILIC RP	273±23 504.1±2.0	8 0.4	0.0001	710±29 771.4±5.7	4 0.7	0.023	252.3±2.8 359.9±3.8	1 1	0.000 1
MA	HILIC RP	N/A 1234.6±8.7	- 0.7	-	N/A 1131±18	- 2	-	N/A 1277.9±8.1	- 0.6	-
EA	HILIC RP	N/A 147.6±2.5	- 2	-	N/A 73.35±0.41	- 1	-	N/A 105.4±4.5	- 4	-
DMA	HILIC RP	N/A 259.6±4.9	- 2	-	N/A 172.0±3.8	- 2	-	182.8±4.5 450.2±4.4	2 1	-
PA	HILIC RP	N/A N/A	- -	-	N/A N/A	- -	-	N/A N/A	- -	-
BA	HILIC RP	N/A 23.69±0.88	- 4	-	N/A 22.5±1.1	- 5	-	N/A 24.8±1.3	- 5	-
DEA	HILIC RP	N/A 13.61±0.34	- 3	-	N/A 13.82±0.71	- 5	-	N/A N/A	- -	-
HEA	HILIC RP	N/A N/A	- -	-	N/A N/A	- -	-	N/A N/A	- -	-
FEA	HILIC RP	N/A 60.8±3.9	- 6	-	N/A 34.08±0.11	- 0.3	-	N/A 34.6±1.7	- 5	-

IZOPA	HILIC RP	N/A 54.6±2.1	- 4	-	N/A 53.2±2.8	- 5	-	N/A 44.9±3.5	- 8	-
SPD	HILIC RP	N/A 1053±11	- 1	-	N/A 1047.1±1.1	- 0.1	-	N/A 1076.8±1.2	- 0.1	-
SPM	HILIC RP	N/A 394±15	- 4	-	N/A 344.5±2.2	- 1	-	N/A 582.4±5.9	- 1	-

—*N/A –not available*

bold P-values > 0.05

Table 4. Recoveries of analytes in six beer samples [%].

BAs	Method	B1 [%]	B2 [%]	B3 [%]	B4 [%]	B5 [%]	B6 [%]
AGM	HILIC RP	79 81	83 80	36 88	75 124	92 92	115 120
PUT	HILIC RP	80 93	58 80	78 104	88 85	97 98	113 93
HIS	HILIC RP	- -	- 106	- 94	- 98	- 99	- 89
TRP	HILIC RP	67 117	37 104	19 106	3 116	99 78	27 110
CAD	HILIC RP	50 82	24 87	107 77	31 84	62 78	80 109
TYR	HILIC RP	73 102	36 93	103 98	28 86	73 101	61 86
MA	HILIC RP	- 100	- 111	- 97	- 94	- 104	- 95
EA	HILIC RP	- 80	- 114	- 97	- 88	- 82	- 106
DMA	HILIC RP	- 79	- 84	- 94	- 90	- 78	18 101
PA	HILIC RP	- -	- -	- -	- -	- -	- -
BA	HILIC RP	- 103	- 83	- 78	- 111	- 92	- 93
DEA	HILIC RP	- 82	- 87	- 88	- 90	- 78	- -
HEA	HILIC RP	- -	- -	- -	- -	- -	- -
FEA	HILIC RP	- 109	- 112	- 94	- 94	- 86	- 109
IZOPA	HILIC RP	- 96	- 97	- 84	- 85	- 94	- 90

SPD	HILIC	-	-	-	-	-	-
	RP	84	95	125	109	118	125
SPM	HILIC	-	-	-	-	-	-
	RP	86	98	114	82	90	112

- Comparison of reversed phase and hydrophilic interaction liquid chromatography
- Application of two chromatographic methods for biogenic amines determination
- Analysis of real samples of beer for biogenic amines content
- Derivatization reaction as necessary step to improve reliability of results

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