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Original Article

The determination of biogenic amines in Turkish red wines

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Abstract

The enological importance of the biogenic amines in wines is due to their possible toxicological risks and the possibility of the relationship between high amine content and unsanitary conditions during wine production procedures. The aim of this work is to determine the biogenic amines profile of quality red Turkish wines by using high-performance liquid chromatography with pre-column derivatization and photodiode array detection. The levels of biogenic amines in Turkish red wines were investigated for the first time. In order to do this, 30 different red wines from four basic wine regions of Turkey (Thrace, Aegean, Central Anatolia and Eastern Anatolia) were analysed for their biogenic amine contents such as tryptamine, putrescine (PUT), histamine (HIST), phenylethylamine (PEA), tyramine (TYR), cadaverine (CAD), spermine (SPM), spermidine (SPD) and agmatine (AGM). Although the majority of the researchers have used and are still using *o*-phytaldialdehyde derivates in separation of these amines, we preferred to use dansyl chloride because of its stability in the UV-Visible detection system. We saw that amines suspected of having toxicological effects (HIST, TYR, and PEA) do not present any concern, as their amount does not exceed 2 mg/L: TYR ranged between 0 and 0.292 mg/L, HIST ranged between 0 and 1.965 mg/L and PEA ranged between 0 and 0.365 mg/L. However, tryptamine (another amine related with toxicological effect) yields variable results that ranged between 1.09 and 7.94 mg/L. The amines associated with deficient sanitary conditions (PUT and CAD) are generally in low amounts: 70% of those samples ranged between 0 and 0.5 mg/L, 16.6% ranged between 0.5 and 1 mg/L, with a maximum of 5.92 mg/L (Aegean) for CAD and 76.6% ranged between 0 and 0.5 mg/L, 6.6% ranged between 0.5 and 1.0 mg/L, 10% ranged between 1 and 2 mg/L with a maximum 3.943 mg/L. In the case of the other amines such as SPD and SPM, they also yielded a very low level: 0–2.186 mg/L for SPD and 0–1.748 for SPM. Only AGM yielded very variable results in our UV-Vis range (0–18.181).

Keywords: Red wines; Biogenic amines; HPLC; PDA

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1. Introduction

Amines in foods are produced mainly by decarboxylation of the amino acids or the transamination of aldehydes and ketones. Biogenic amines can be found in various foods and beverages such as wine, beer, fish and meat, normally as a result of the enzymatic degradation or the fermentation processes. But they are often found in fermented foods such as cheese, dry sausage and red wine (Kirschbaum et al., 1994; Ibe et al., 1991). Biogenic amines can cause direct or indirect toxicity when their concentration levels are high (Aerny, 1990). For this reason some countries have established regulations for their intake content in various kinds of foods (Busto et al., 1997; Maijala, 1994). USA, Sweden, Austria and Netherlands have established regulations and legal requirements for the maximum limits of biogenic amines (mainly histamine (HIST)) in various foods. The lack of legislation on the tolerated contents of biogenic amines in wine makes it difficult to import and export this product (Busto et al., 1995; Tricard et al., 1991). There may be three possible origins of biogenic amines in wines: they are already present in the must; they are formed by the yeast during the alcoholic fermentation, and/or they are formed in wine by the action of bacteria involved in the malolactic fermentation (MLF) (Arce et al., 1998; Vidal-Carou et al., 1990). The formation of biogenic amines in fermented foods is related to the presence of certain micro-organisms which are able to produce decarboxylase under appropriate conditions and proper cofactors when appropriate amino acids are present (Leitão et al., 2000). It is obvious that biogenic amines have toxic effects. The toxic limits for biogenic amines are given as follows in terms of HIST: 8–40 mg causes slight poisoning, 40–100 mg, intermediate, over 100 mg can cause intensive poisoning; also tyramine (TYR) contents over 100 mg can cause migraine (Maijala and Eerola, 1993; Ayhan et al., 1999). In determining biogenic amines several methods have been used by different scientists. Frequently, these methods are based on molecular spectroscopic techniques. But when several amines are present in foods, thin-layer chromatography, gas chromatography, mass spectrometry, liquid chromatography or capillary electrophoresis are generally employed. Current reference and analytical methods use high-performance liquid chromatography (HPLC) (Busto et al., 1995). A major problem with biogenic amines identification and quantification in wines is the lack of a sensitive analytical technique. Different derivatizing agents and/or other sample treatments are still used to overcome the matrix complexity problems in wine, especially in red wines. In order to prevent matrix interference and to achieve enhanced detection sensitivity, derivatizing reagents dansyl chloride (Dns-Cl) (Busto et al., 1995, 1997) and *o*-phytaldialdehyde (OPA) are commonly used in the presence of 2-mercaptoethanol. Phenyl isothiocyanate, fluorenyl methyl chloroformate (FMOC-Cl), benzyl chloride and fluorescamine are the other derivatizing agents used in these determinations (Kirschbaum et al., 1994; Ibe et al., 1991; Busto et al., 1996; Pfeiffer and Radler, 1992). Although OPA is used successfully in fluorimetric detection because it reacts with primary amines in a few minutes and forms strong fluorescent derivates which increase the method selectivity (Gerbaux et al., 1997), they are not very stable, they do not allow clean-up after the derivatization processes and they need strict derivatization conditions (Mafra et al., 1999; Buteau et al., 1984a, b). Dns-Cl is a stable reagent especially in UV-Vis detection systems. In this study, we used Dns-Cl as a derivatization reagent with gradient elution. Nine different biogenic amines were rapidly determined in Turkish quality wines by using HPLC with pre-column derivatization and photodiode array (PDA) detection.

2. Materials and methods

2.1. Samples

Thirty red wines were selected from four different regions of Turkey, namely Aegean, Thrace, Central Anatolia and Eastern Anatolia. The wine samples were taken from 10 different wineries (small, medium and large producers). In all cases, samples were taken throughout the entire production of each winery.

2.2. Analytical methods

Total sulphur dioxide, total and volatile acidity, alcohol content, and pH were determined by following the OIV (Office International de la Vigne et du Vin; OIV Recueil des Methodes Internationales d'Analyse des Vins, 1969) methods and by following the "Cahiers des Travaux Pratiques (1988) (Université Victor Ségalen Bordeaux II)" methods. L-lactic acid and L-malic acid were determined by using enzymatic methods (Riberau-Gayon et al., 1982).

2.3. Reagents

Biogenic amine standards: tryptamine (TRP), phenylethylamine (PEA), putrescine (PUT), cadaverine (CAD), HIST, TYR, spermidine (SPD), spermine (SPM) and agmatine (AGM) were from SIGMA, acetonitrile from Merck (gradient grade), Tris-(hydroxymethyl)-aminomethan from Fluka AG, Busch SG, sodium glutaminate (L(+)-glutamic acid monosodium salt monohydrate 99%) from Acros Organics, sodium carbonate anhydrous from J.T. Barker, Dns-Cl from Sigma. Derivatization with Dns-Cl realized as follows: 10 mg Dns-Cl was made up in 1 mL acetone, 2 g Na_2CO_3 was made up in 10 mL and 200 mg sodium glutaminate in 4 mL mQ water. Tris-(hydoxymethyl)-aminomethan were used for the preparation of the buffer in the mobile phase. The adjustment of pH was made with acetic acid (pure). Buffer, acetonitrile and water (MQ) were used for the mobile phase. All solutions were freshly prepared.

2.4. Chromatographic system and equipment

The system included an HPLC; Shimadzu LC-10 AD pump, SPD-M10AVP Diode Array detector (200–550 nm), CTO-10 A Column Oven. 50 μL of sample loop with Reodayn Walve model 7725i Manual Sample Injection and CBM-10A Communications Bus Module. As chromatographic column, Phenomenex Luna 5u RP-18 (250 × 4.6 mm) was used. The centrifugation made with Labofuge 200 Hareus Sepatech centrifuge delivered with an integrated angle rotor (max speed 5300 rpm: 3030*g*).

2.5. Preparation of stock standard solutions

Amine standard solutions were prepared in 0.4 M perchloric acid to a final concentration of 10 mg/50 mL for each biogenic amine. For analyses, 5 mL of each stock solution was diluted to 50 mL with 0.4 M perchloric acid.

2.6. Preparation of samples

About 5 mL of each wine was homogenized for 30 min in an ultrasonic bath. The wine samples were filtered by Millex-LCR 13 mm, 0.5 μm filter before derivatization.

2.7. Derivatization procedure of sample extracts and mixed standards

Exactly 400 μL of sodium carbonate solution was added to 400 μL of the extract, mixed with 400 μL Dns-Cl and held at 40°C in a water bath for 30 min to remove residual Dns-Cl, 200 μL sodium glutaminate was added and the solution held at 40°C for further 60 min. After adding 1 mL of acetonitrile, the samples were centrifuged with Labofuge 200 Heraeus Sepatech centrifuge delivered with an integrated angle rotor (max speed 5300 rpm: max RCF 3030*g*) at 1250 rpm (715*g*) for 20 min and the supernatant filtered through a 0.45 μm filter. Dansyl derivatives of the calibration standards were mixed with the samples as previously described (Nouadje et al., 1997; Pereira Onterio and Bertrand, 1994).

2.8. Chromatographic conditions

Buffer: pH = 8
[0.1 M tris-(hydroxymethyl)-aminomethan/0.1 M acetic acid/water(MQ): (2/1/2)]
Two solvent reservoirs containing:
Solution A: buffer (30 mL) + acetonitrile (550 mL) + water (420 mL)
Solution B: buffer (2 mL) + acetonitrile (900 mL) + water (100 mL)

An initial linear gradient elution program was used to separate all amines (Table 1, Figs. 1 and 2).

2.9. Statistical analysis

Statistical analysis results were obtained by using multiple analysis of variance (Sokal and Rohlf, 1995).

3. Results and discussion

Our purpose was to determine the kinds of biogenic amines in Turkish red wines from different Anatolian wine regions by HPLC, using Dns-Cl as derivatizing agent. Table 2 shows the results of the concentration of biogenic amines of Turkish red wines and Table 3 the linearity of SPD response at 220 nm wavelength. The analysis of 30 red wine samples shows that Turkish quality red wines generally contain small quantities of biogenic amines and those levels are acceptable in European countries. There has been a lot of research on the biogenic amine content of commercial wines. In France; 54 red, 15 rosé and 15 white commercial bottled wines from Vallée du Rhône have been analysed with HPLC (FMOC derivatization) to determine their amine content such as HIST, TYR, PEA, PUT and CAD. Only AGM and PUT level were found to be higher than 1 mg/L

Table 1
The gradient program used in chromatography, initial linear gradient elution, total flow 1.3 mL/min

Time (min)	Solution B (%)	Solution A (%)
0.1	5	95
10	10	90
15	15	85
20	25	75
25	63	37
30	100	—
40	5	95

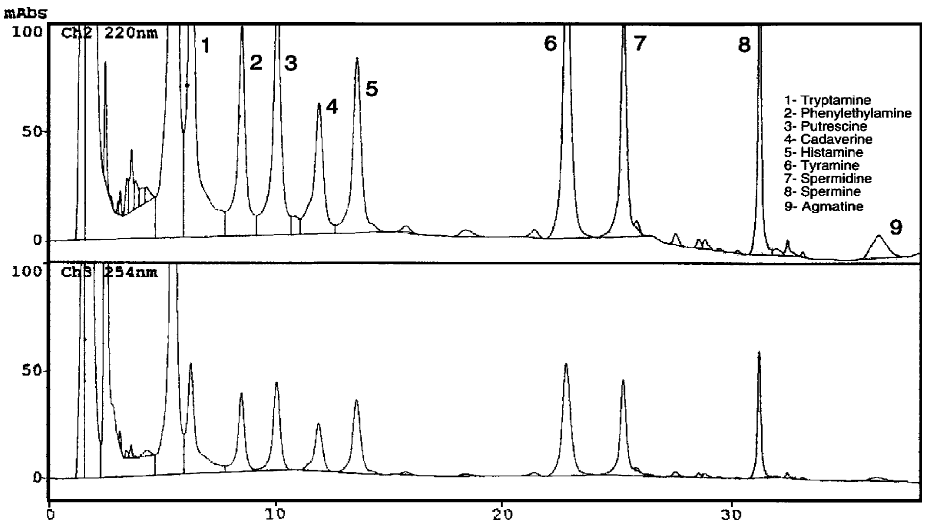

Fig. 1. Chromatogram of a standard solution.

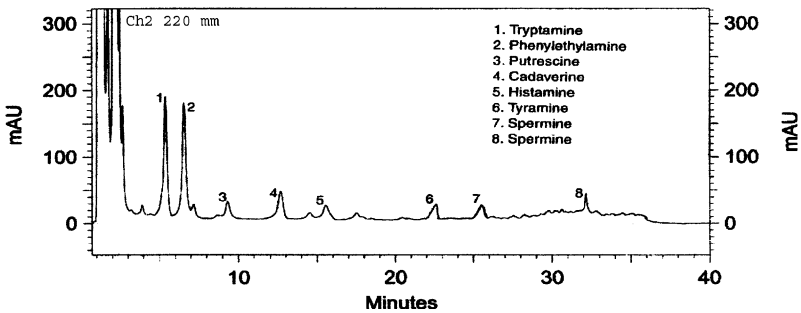

Fig. 2. Example of red wine processed with the analytical procedure described.

(8% of the samples contained more than 20 mg/L of PUT, 1.2% more than 10 mg/L of HIST and TYR) (Bauza et al., 1995). The biogenic amines content of 109 different commercial Rioja D.O.C. wines was determined with HPLC (OPA derivatization) and the highest amine content was found

Table 2
Concentration of biogenic amines [mg/L] ($n = 3$, all parameters are given with their standard error)

Wine	TRP	PUT	PEA	CAD	HIST	TYR	SPD	SPM	AGM
Aegean									
1A	1.093±0.0179	5.920±0.0300	2.362±0.0397	1.850±0.0335	1.965±0.0240	ND	2.186±0.0263	0.290±0.0084	2.297±0.0362
2A	2.707±0.0223	ND	0.203±0.0049	0.142±0.0029	ND	0.129±0.0006	0.057±0.0007	0.0765±0.0015	0.5602±0.0009
3B	4.066±0.0032	0.070±0.0030	0.296±0.0012	0.507±0.0032	ND	0.060±0.0006	0.300±0.0012	0.817±0.0112	9.136±0.0188
4B	5.775±0.0086	1.885±0.0018	3.873±0.0095	0.344±0.0015	0.180±0.0009	ND	ND	ND	18.181±0.1521
5B	5.667±0.0516	0.587±0.0006	0.334±0.0015	0.157±0.0009	0.225±0.0012	0.096±0.0003	0.054±0.0009	0.888±0.0031	11.625±0.003
6B	2.528±0.0019	0.103±0.0003	0.111±0.0007	0.191±0.0006	0.043±0.0003	0.030±0.0006	0.317±0.0032	0.449±0.003	4.107±0.0125
7C	6.070±0.0041	0.449±0.0005	1.208±0.0025	0.132±0.0009	0.049±0.0009	ND	ND	0.396±0.0041	0.348±0.0030
8C	4.770±0.0044	ND	ND	ND	1.004±0.0009	ND	ND	0.396±0.0041	0.348±0.0030
9C	3.717±0.0041	0.314±0.0009	2.038±0.0150	ND	ND	ND	ND	ND	1.802±0.0083
10C	1.959±0.0044	ND	0.038±0.0007	ND	ND	ND	ND	0.732±0.0009	3.556±0.024
Thrace									
11D	4.682±0.0100	0.296±0.0006	0.324±0.0015	0.267±0.0009	0.144±0.0032	0.229±0.0032	0.574±0.0041	0.876±0.0044	ND
12D	4.944±0.0220	0.724±0.0026	ND	2.429±0.0059	0.372±0.0041	0.077±0.00	0.328±0.0018	ND	5.536±0.0200
13E	3.821±0.0121	0.874±0.0032	ND	ND	ND	ND	ND	0.754±0.0136	3.745±0.0173
14E	4.481±0.0116	0.205±0.0035	0.250±0.0020	0.041±0.0009	0.059±0.0029	ND	ND	0.675±0.0026	ND
15E	5.324±0.0071	0.499±0.0026	0.536±0.0012	1.004±0.0038	ND	0.157±0.0062	0.070±0.0015	ND	ND
16F	0.863±0.0092	ND	ND	ND	ND	ND	ND	0.106±0.0038	0.926±0.0365
17G	5.141±0.0139	2.769±0.0254	0.331±0.0015	0.901±0.0015	0.910±0.0038	ND	0.9727±0.0021	0.195±0.0012	8.491±0.0280
18G	4.326±0.0151	1.123±0.0171	0.176±0.0023	0.079±0.0009	0.197±0.0009	0.200±0.0029	0.286±0.0022	0.110±0.0033	ND
19G	4.094±0.0111	0.632±0.0171	0.048±0.0020	0.217±0.0035	0.165±0.0029	ND	ND	0.069±0.0020	ND
20G	7.137±0.0116	ND	ND	ND	ND	0.132±0.0015	0.382±0.0042	0.352±0.0023	ND
Central Anatolia									
21H	4.228±0.0104	0.080±0.0003	0.084±0.0020	ND	0.060±0.0041	0.038±0.0003	0.338±0.0068	0.158±0.0009	ND
22H	5.508±0.0140	0.455±0.0018	0.415±0.3130	ND	ND	ND	ND	0.448±0.056	ND
23H	5.317±0.0115	ND	ND	1.766±0.0326	0.613±0.0020	0.043±0.0003	0.678±0.0029	1.748±0.0066	ND
24H	7.944±0.0382	0.113±0.0852	ND	0.061±0.0003	0.280±0.0023	0.110±0.0006	0.013±0.0007	0.087±0.0015	0.041±0.0021
Eastern Anatolia									
25J	2.938±0.0193	0.292±0.0064	0.026±0.0033	0.109±0.0006	0.514±0.0029	0.035±0.0006	ND	0.439±0.0078	ND
26J	3.181±0.0205	0.670±0.0020	0.092±0.0015	0.443±0.0033	ND	ND	ND	0.326±0.0012	0.097±0.0044
27J	5.417±0.0489	0.426±0.0038	ND	ND	ND	0.060±0.0015	ND	0.090±0.0112	ND
28J	3.613±0.0122	ND	0.067±0.0022	ND	ND	0.025±0.003	ND	ND	ND
29K	ND	ND	3.365±0.0394	3.943±0.0381	ND	ND	ND	ND	ND
30K	5.632±0.0171	ND	ND	0.126±0.0059	ND	ND	0.087±0.0043	ND	ND

A, B, C, D, E, F, G, H, J, K: same letters signify same wineries of each region.
Concentration range: 0.4–8.8 mg/L (TRP), 0.94–15.6 mg/L (PUT), 0.10–8.6 mg/L (PEA), 0.11–4 mg/L (CAD), 0.03–2.8 mg/L (HIST), 0.04–3.5 mg/L (TYR), 0.015–1 mg/L (SPD), 0.05–3.4 mg/L (SPM), 0.5–15 mg/L (AGM).

Table 3
Linearity of SPD (photodiode array detector) response at 220 nm wavelength

Biogenic amines	Code	Intercept	Slope	R^2
Tryptamine	TRP	−3.254E−06	9.806E+09	0.9995
Phenylethylamine	PEA	8.552E−06	4.133E+09	0.9994
Putrescine	PUT	1.092E−06	4.524E+09	0.9997
Cadaverine	CAD	3.354E−06	3.082E+07	0.9995
Histamine	HIST	1.681E−06	3.995E+09	0.9998
Tyramine	TRY	−9.812E−06	5.079E+09	0.9978
Spermidine	SPD	3.939E−07	4.082E+09	0.9996
Spermine	SPM	−4.959E−06	2.857E+09	0.9983
Agmatine	AGM	−0.0001008	4.880E+07	0.9818

in red wines. The maximum level was 33.10 mg/L for PUT, 1.74 mg/L for CAD, 5.98 mg/L for TYR and 8.72 mg/L for HIST in analysed red wines (Vazquez-Lasa et al., 1998). Thirty Portuguese wines (including fortified wines such as Port) have been analysed with HPLC (OPA derivatization); the maximum content was found 1.7 mg/L for HIST. PUT and CAD yielded very low level varying between 0.2 and 0.6 mg/L (Mafra et al., 1999).

Regarding all the wine regions of Turkey, the level of the amines is generally very low. Aromatic and heterocyclic amines that have toxicological effects (HIST, TYR, TRP and PEA) were: 0–0.292 mg/L for TYR, 0–1.965 mg/L for HIST, 0–0.365 mg/L for PEA and 0–7.994 mg/L for TRP. These values are generally very low, especially for TYR, HIST and PEA (100% of the samples for TYR, 83.3% of the samples for HIST and 80% of the samples for PEA contained less than 0.5 mg/L for each amine). However, TRP gives variable results between 1.09 and 7.94 mg/L. The amines associated with sanitary conditions (PUT and CAD) are also found to have very low ranges; 70% ranged between 0 and 0.5 mg/L, 16.6% ranged between 0.5 and 1.0 mg/L, with a maximum level of 5.92 mg/L for CAD and 76.6% 0–0.5 mg/L, 6.6% 0.5–1 mg/L, 10% 1–2 mg/L, with a maximum level of 1.85 mg/L. Only AGM gives very variable results in our UV-Vis range (0–18.181). Comparing the results for the wine regions of Turkey by variance analysis techniques, there are no significant differences and correlation between the regions and the different wineries.

When the total amounts of biogenic amines are compared, it was found that some samples contained quite high levels of amine (Table 2). Certainly, red wines having undergone MLF contain higher levels of amine, particularly HIST (Buteau et al., 1984a, b). In our study the majority of red wines had already undergone MLF. However, in the samples containing malic acid (three samples of Aegean, one sample of Central Anatolia, four samples of Eastern Anatolia, in total eight samples) (Table 4), the level of some amines seems somewhat lower than the others such as: tryptamine in Thrace (sample 20) and Eastern Anatolia (sample 27, 28). But it is impossible to give any correlation regarding these data.

The use of Dns-Cl as a derivatizing reagent seems to be an alternative choice for OPA and in our conditions, yields satisfactory results for the determination of the biogenic amines (except AGM which does not yield a good level of stability in our UV-Vis range, probably due to the rapid decomposition of this amine). This method also gives good linearity of SPD at 220 nm with acceptable R^2 values (Table 3). The results of biogenic amine analysis, linearity and R^2 values are also given for AGM, despite the lack of satisfactory results and stability in these conditions.

Table 4
Enological parameters of Turkish red wines

Wine	Alcoholic content (v/v)	Total SO_2 (mg/L)	pH	Total acidity (g/L)	Volatile acidity (g/L)[a]	Malic acid (g/L)[b]	Lactic acid (g/L)
Aegean							
1	12.5	187	4.21	5.32	0.43	[c]	0.81
2	12.7	175	4.11	5.13	038	[c]	0.87
3	12.1	168	4.13	5.34	0.46	[c]	0.76
4	13.0	145	3.93	5.19	0.56	[c]	0.78
5	12.9	184	3.98	5.27	0.43	[c]	0.90
6	11.7	199	4.14	5.42	0.34	1.11	0.20
7	11.5	210	3.78	5.22	0.39	[c]	1.23
8	12.1	182	3.81	5.19	0.54	[c]	1.14
9	11.9	178	3.65	4.95	0.30	1.27	[c]
10	12.4	190	4.05	5.33	0.45	1.34	[c]
Thrace							
11	11.7	178	3.98	5.11	0.47	[c]	0.98
12	12.9	182	3.92	5.08	0.60	[c]	0.87
13	12.7	190	3.82	5.16	0.56	[c]	0.76
14	12.1	156	3.90	4.13	0.53	[c]	1.21
15	13.1	187	3.94	4.38	0.43	[c]	1.26
16	13.4	201	3.87	4.87	0.36	[c]	1.19
17	11.9	154	3.91	5.0	0.32	[c]	1.02
18	12.7	165	3.65	5.19	0.39	[c]	0.90
19	13.0	197	3.75	4.87	0.41	[c]	0.87
20	11.7	217	3.69	4.80	0.42	1.17	0.19
Central Anatolia							
21	11.8	201	3.65	4.95	0.54	[c]	0.87
22	12.1	219	3.72	4.80	0.47	1.26	[c]
23	12.4	198	3.49	5.31	0.39	[c]	1.25
24	12.5	189	3.72	5.22	0.36	[c]	1.14
Eastern Anatolia							
25	11.8	143	3.82	4.82	0.52	[c]	1.40
26	12.2	187	3.96	4.61	0.45	1.52	[c]
27	12.6	115	3.66	4.81	0.37	[c]	1.07
28	12.1	195	3.94	4.32	0.42	1.33	0.22
29	12.4	190	4.0	4.69	0.43	1.45	[c]
30	12.8	234	3.87	4.69	0.46	1.37	[c]

[a] Expressed in tartaric acid.
[b] Expressed in acetic acid.
[c] Less than 0.05 g/L.

4. Conclusions

Dns-Cl reagent is a good alternative for determining biogenic amines in wines and can be used instead of OPA with PDA by pre-column derivatization. However, AGM did not give a stability in our UV-Vis range compared to the others (Tables 2 and 3), although consistently fresh standards were used. The analysis of 30 samples showed that Turkish red wines contain small quantities of biogenic amines (Table 2). However, quite large differences may occur between different samples from different wine regions (Table 3).

With regard to all wine regions of Turkey, the level of the amines are generally very low. Aromatic and heterocyclic amines that have toxicological effects (HIST, TYR, TRP and PEA) were: 0–0.292 mg/L for TYR, 0–1.965 mg/L for HIST, 0–0.365 mg/L for PEA and 0–7.994 mg/L for tryptamine. These values are generally very low, especially for TYR, HIST and PEA (100% of the samples for TYR, 83.3% of the samples for HIST and 80% of the samples for PEA contain less than 0.5 mg/L for each amine). However, TRP gives variable results between 1.09 and 7.94 mg/L. The amines associated with sanitary conditions (PUT and CAD) are also found in very low ranges; 70% ranged between 0 and 0.5 mg/L, 16.6% ranged between 0.5 and 1 mg/L, with maximum level 5.92 mg/L for CAD and 76.6% ranged between 0 and 0.5 mg/L, 6.6% ranged between 0.5 and 1 mg/L, 10% ranged between 1 and 2 mg/L, with maximum level 1.85 mg/L. Only, AGM yielded very variable results in our UV-Vis range (0–18.181). Comparing the results for the wine regions of Turkey with variance analyses techniques, there are no significant differences and significant interaction between the regions and the different wineries.

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