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Derivatization, stabilization and detection of biogenic amines by cyclodextrin-modified capillary electrophoresis–laser-induced fluorescence detection

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Abstract

o-Phthalaldehyde (OPA) derivatives of eight biogenic amines were stabilized at 5°C by forming inclusion complexes with methyl-β-cyclodextrin (MBCD). The derivatives were separated and detected by cyclodextrin-modified capillary electrophoresis (CE) with UV or laser-induced fluorescence (LIF) detection. Using a borate buffer, pH 9.0 consisting of ethanol and a mixture of negatively charged sulfobutylether-β-cyclodextrin and neutral MBCD, baseline separation of the eight OPA derivatives was achieved within 25 min with high separation efficiencies. The detection limits ($S/N=3$) obtained by UV and LIF detection were determined to be 10 μ*M* and 0.250 μ*M*, respectively. Glutamic acid was added after the initial derivatization step to neutralize residual OPA which otherwise caused a significant interference, particularly when analysis was performed around the detection limit of the OPA derivatives. Important biogenic amines in fish, wine and urine were then derivatized and determined by CE–LIF. In the case of sole and rainbow trout, the results obtained were validated by an enzymatic assay using putrescine oxidase.

Keywords: Derivatization, electrophoresis; Food analysis; Biogenic amines; Putrescine; Cyclodextrins; Cadaverine; Histamine

1. Introduction

Biogenic amines are accumulated in foodstuffs and beverages during improper storage as a result of microbial/enzymatic degradation of amino acids. Putrescine, cadavarine and histamine have been implicated in human poisoning when present at considerable concentrations [1–3]. Symptoms such as headaches, nausea, hypo- or hypertension and cardiac palpitations have been reported as a result of excessive intake of biogenic amines, especially histamine [4–6]. The toxic effects of biogenic amines are accelerated by monoamine oxidase inhibitor drugs as well as alcoholic beverages since ethanol is an inhibitor of monoamine oxidase [7,8]. Tyramine, tryptamine and 2-phenylethylamine with vasoactive properties should be limited in foods and food products [9]. Putrescine and cadavarine have been proposed as possible cancer markers as their elevated levels have been found in the serum and urine of cancer patients [10,11].

The biogenic amines of alcoholic beverages and

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wines have been analyzed by high-performance liquid chromatography (HPLC) after derivatization with dansyl chloride [12], dabsyl chloride [13], fluorenylmethylchloroformate [14] and *o*-phthalaldehyde (OPA) [15]. The biogenic amines in fermented foods and biological samples were also derivatized using 3,5-dinitrobenzoyl chloride [16] and trifluoroacetylacetone, respectively [17]. In general, the above procedures are time-consuming with the exception of the OPA derivatization method. However, the instability of the OPA derivatives must be overcome by rather rigorous HPLC methods involving pre-column [18–20], post-column [21] and on-column derivatizations [15]. Factors affecting the stability of the OPA derivatives include the effect of temperature [20] and the choice of various thiols [22].

Capillary electrophoresis (CE) procedures have been developed for analysis of derivatized biogenic amines. Borate buffer containing sodium dodecyl sulfate (SDS) and acetonitrile was capable of separating seven biogenic amines derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate [23]. A similar approach was reported for the measurement of biogenic amines in sauerkraut using benzoyl chloride as derivatizing reagent [24]. Micellar electrokinetic chromatography with laser-induced fluorescence (LIF) detection was also developed for detection of biogenic amines and amino acids derivatized with fluorescein isothiocyanate as an indicator of wine aging [25] and the quality of dairy products [26]. However, CE is not suitable for analysis of unstable OPA derivatives without a complicated on-line derivatization scheme and instrumentation. To our knowledge, cyclodextrin-modified CE has not been attempted for analysis of biogenic amine derivatives.

In this study, the OPA derivatives of biogenic amines were effectively stabilized by forming inclusion complexes with methyl-β-cyclodextrin (MBCD). Cyclodextrin-modified CE coupled with LIF detection has been developed for analysis of such OPA derivatives. The separation scheme was optimized with respect to running buffer pH, cyclodextrin concentration, ethanol concentration, temperature and separation potential. Glutamic acid was necessarily added after derivatization to neutralize residual OPA that caused a significant interference. The method was then applied for the determination of biogenic amines in fish, wine and urine with its applicability validated by enzyme assays.

2. Experimental

2.1. Materials

The biogenic amine standards with highest purity, peroxidase, Brij 35, 2-mercaptoethanol, EDTA, glutamic acid, and OPA were purchased from Sigma (St. Louis, MO, USA). The stock solutions of the amines (50 m*M*) were prepared in water and stored at 5°C. MBCD (degree of substitution, DS, 1.6–2.0, average molecular mass: 1310), hydroxypropyl-β-cyclodextrin (HPCD) and 4-aminoantipyrine were obtained from Aldrich (Milwaukee, WI, USA). Sulfobutylether-β-cyclodextrin (SBCD, DS 5.5, average molecular mass 2005) and putrescine oxidase were purchased from CyDex (Overland Park, KS, USA) and Toyoba (Osaka, Japan), respectively. HPLC-grade acetonitrile (ACN) and reagent ethanol were obtained from Fisher (Nepean, Canada) and the remaining chemicals were purchased from Anachemia (Montreal, Canada). Samples of sole, rainbow trout, red and white wines were purchased from local markets, and urine was collected from a healthy subject.

2.2. Instrumentation

CE experiments were performed on a P/ACE 5500 capillary electrophoresis system (Beckman, Fullerton, CA, USA). Fused-silica capillary (50 μm I.D.) was purchased from Polymicro Technologies (Phoenix, AZ, USA) and cut to an overall length of 47 cm with an effective length of 40 cm from the inlet to the detector window. On-column UV detection of the derivatized biogenic amines was performed with a Beckman modular UV detector operated at 214 nm. The capillary temperature was regulated at 25°C using a liquid coolant in a sealed cartridge. The separation of the derivatized biogenic amines was also monitored using the Beckman LIF detector module. The 325 nm, 35 mW output of an Omnichrome series 74 He–Cd laser (Melles Griot, Carlsbad, CA, USA) was coupled to a 100/140 μm

core/cladding size fused-silica step-index optical fiber (OZ Optics, Ottawa, Canada, transmission yield of 85–90%). Fluorescence emission was monitored using a 420 nm long-pass filter coupled with a 450 nm bandpass filter before the photomultiplier tube in the LIF detector.

The fluorescence response of the OPA derivatives under the above operating conditions was 80% of that obtained at the optimal conditions (excitation 350 nm, emission 450 nm) as determined using the Gilford Fluoro-IV spectrofluorometer (Gilford, Oberlin, OH, USA). Capillary temperature was regulated at 15°C in the case of fluorescence experiments. Sample injection was performed by applying a small pressure (0.5 p.s.i., 3.44 kPa) at the inlet of the capillary for 5 s. The separation voltage (+25 kV for UV detection and +20 kV for fluorescence detection) was applied over a 1 min ramp to prevent possible current breakdown. Between runs the capillary was washed with a cycle including 3 *M* NaCl, followed by ACN, 1 *M* HCl, 1 *M* NaOH, 0.1 *M* NaOH, water and finally the running buffer. This washing procedure greatly improved reproducibility from run to run especially for the spermine and spermidine OPA derivatives. Increased fluorescence detection limits were attempted using an extended light path capillary (50 μm I.D., 250 μm I.D. at the detector window and 360 μm O.D., Agilent, Mississauga, Canada) installed in a capillary cartridge.

Data acquisition and analysis were facilitated by using P/ACE Station software (Version 1.21, Beckman). The resolution (R_s) of the peak from the preceding peak was calculated as $1.18\cdot(t_2-t_1)/(w_{1/2,1}+w_{1/2,2})$, where t_i and $w_{1/2,i}$ represent the migration time of the analyte and the full peak width at half-maximum, respectively [27]. Separation efficiency was defined as the number of theoretical plates (N) calculated as $5.54\cdot(t_i/w_{1/2,i})^2$ where t_i and $w_{1/2,i}$ are defined as above. The theoretical plate number was divided by the effective capillary length (40 cm) to obtain the number of theoretical plates per meter (N/m).

2.3. Derivatization of biogenic amines

The OPA derivatives of the biogenic amines were prepared as described by Chemnitius and Bilitewski [28], with some modifications. A 5-g amount of boric acid, 4.4 g potassium hydroxide, 0.3 g Brij 35, 0.3 ml 2-mercaptoethanol and 80 mg OPA (6 m*M*) in 100 ml of distilled water was used as derivatizing reagent. The effects of light, cyclodextrins, and temperature on the stability of the derivatives (2 μ*M* and 2 m*M*) were monitored using the spectrofluorometer (excitation, 350 nm and emission, 450 nm) with the photomultiplier tube (PMT) set at 600 or 400 V. For the preparation of the derivatives at 2 μ*M*, the OPA concentration used was 200 μ*M*.

With samples prepared for CE analysis, the derivatization procedure was altered slightly by replacing Brij 35 in the derivatizing reagent by 100 m*M* MBCD. Owing to the instability of 2-mercaptoethanol in the borate buffer at pH 10.7, 1 m*M* EDTA was added to stabilize the reaction mixture. OPA (200 m*M*) prepared in ethanol was added to the derivatizing reagent just before reaction with the biogenic amine standard (final concentration, 50 μ*M* each for fluorescence or 1–2 m*M* for UV detection) or the samples. The final concentrations of OPA in the derivatization reaction (400 μl, containing 20 μl sample) were 16 and 4 m*M*, respectively, for UV and fluorescence detection. Both of these reagents were prepared fresh daily. In CE–LIF experiments, unreacted OPA was derivatized by the addition of excess (10 m*M*) glutamic acid (4 μl, 1 *M* stock solution).

2.4. Sample preparation

As described by Male et al. [29], fish extracts were prepared by homogenizing 5 g of fish fillet with 10 ml of 10% trichloroacetic acid. After centrifugation, the supernatants (11–13 ml) were neutralized by adding 200 m*M* borate, pH 9.0 (2 ml) and 500 μl of 8 *M* NaOH. Fillets were left at 5°C, or room temperature and sampled periodically to provide various concentrations of the analytes as the level of biogenic amines has been known to increase during the course of storage [29]. The samples (20 μl) were then derivatized as above and monitored by CE. Red and white wine samples (100 μl) could be monitored by CE directly without any treatment. Urine samples were hydrolyzed as described by Kubota et al. [30] with minor modifications. Urine (3 ml) was diluted 1:1 with concentrated HCl and heated at 100–110°C for 16 h in an oil bath. After centrifugation, the

collected supernatant was neutralized with 8 *M* NaOH and diluted twofold in 200 m*M* borate, pH 9.0. The putrescine in the sample (200 μl) hydrolyzed from acetylputrescine was then detected by CE.

2.5. Enzymatic assay for putrescine and cadavarine in fish

Putrescine and cadavarine in fish samples were monitored using putrescine oxidase coupled to a standard hydrogen peroxide detection method using 4-aminoantipyrine–phenol–peroxidase [31]. The assay buffer (25 m*M* borate, pH 8.0) contained 2 m*M* phenol, 0.5 m*M* aminoantipyrine, 10 units of peroxidase and fish extract (50–250 μl). After adding 1 unit of putrescine oxidase to the metabolite assay (1 ml), the formation of the red quinoneimine dye was monitored at 505 nm (DU 640, Beckman). Unlike putrescine, the putrescine oxidase reaction with cadavarine was much slower, therefore, putrescine concentrations could be reliably estimated from the absorbance change after 1 min, whereas total putrescine plus cadavarine was monitored after 60 min.

3. Results and discussion

3.1. Stabilization of o-phthalaldehyde derivatives of biogenic amines

As expected, the OPA derivatives of all eight biogenic amines were very unstable at room temperature. Within the first 5 h over 35 and 90%, respectively, of the fluorescent signal obtained for the cadavarine and histamine derivatives at 2 m*M* had been lost (Fig. 1). Both tyramine and putrescine derivatives mimicked the histamine derivative, while the responses of spermine, spermidine, tryptamine and phenylethylamine derivatives were similar to that of the derivatized cadavarine. Performing the derivatization at 5°C greatly improved the stability of the OPA derivatives as illustrated in Fig. 1. In this case, only 10 and 40% of the fluorescence signal for cadavarine and histamine, respectively, was lost within 5 h. The stability was not affected by light

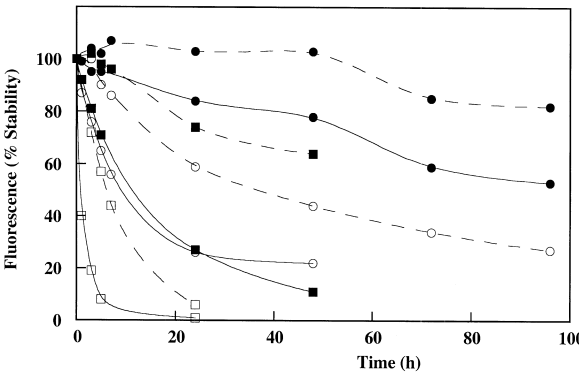

Fig. 1. Effect of temperature; 5°C (– – –) and 25°C (—) on the stability of OPA derivatives of cadavarine (○, ●) and histamine (□, ■) at 2 m*M*, in the presence (●, ■) and absence (○, □) of 100 m*M* MBCD.

and the reaction was virtually instantaneous (within 30–60 s) regardless of the reaction temperature.

The solubility of the derivatives at the concentration used in this study was fairly low despite the presence of the surfactant Brij 35. Cyclodextrins are known to enhance the solubilization of bulky water-insoluble compounds that are able to enter and/or interact with the cyclodextrin cavity, and in certain cases enhancement of stability of the guest compound is also noted [32]. MBCD was observed to improve the solubility of all eight biogenic amine derivatives as well as enhance their stability at room temperature (Fig. 1). During the first 5 h, only 5% of the fluorescence signal obtained for the cadavarine derivative was lost compared to 30% of the histamine derivative. The presence of 100 m*M* cyclodextrin significantly improved the stability for cadavarine, putrescine and histamine, while this beneficial effect for the other five derivatives was somewhat marginal. The stability improvements noticed with MBCD were found to be superior to those obtained with HPCD.

Notice also that performing the reaction in the presence of cyclodextrins increased the absolute fluorescence of all the derivatives, presumably due to the enhanced solubility. The combination of low temperature and the addition of 100 m*M* MBCD provided the best result (Fig. 1). The cadavarine derivative was completely stable for the first 48 h while the fluorescence signal for the histamine

derivative decreased only 5 and 25% during the first 5 and 24 h, respectively. The results obtained for tyramine were similar to cadavarine, while those of putrescine, tryptamine and phenylethylamine mimicked the histamine results. The most instability was noted for spermine and spermidine. Nevertheless, all derivatives only lost less than 10% during the first 5 h. Therefore, such derivatives were stable enough to be processed by CE without having to encounter the inherent difficulties and complexities of on-line derivatization.

The derivatization reaction performed at 2 μ*M* biogenic amine displayed a similar stability pattern compared to that of 2 m*M*, although the effect was less pronounced. For example, at room temperature in the absence of cyclodextrin the derivative for cadavarine lost about 5% of its fluorescence in 4 h while 55% was lost in the case of histamine. As noted earlier, the derivatization was performed at 5°C in the presence of 100 m*M* MBCD to enhance the stability such that all derivatives were completely stable for at least 24 h. This was significant since LIF detection of the derivatives using CE would be performed in this concentration range.

3.2. Separation of OPA derivatives by capillary electrophoresis using UV detection

The separation of derivatives of the biogenic amines with CE was first attempted using UV detection. In this experiment only cadavarine, phenylethylamine, tyramine, histamine and tryptamine were used since each of the other three derivatives yielded multiple peaks in the UV. All samples were run within 5 h of the derivatization reaction to maintain stability. Baseline separation was achieved under the following conditions: 50 m*M* borate buffer, pH 9.0, containing 25 m*M* SBCD, 20 m*M* MBCD, and 10% ethanol at +25 kV and 25°C (Fig. 2).

The running buffer pH was noted to affect the order of migration. At pH 11 the tyramine peak shifted across the histamine and tryptamine peaks and emerged as the last one, with a total separation time of 70 min instead of 25 min. Likewise, altering the ratio of the two cyclodextrins also affected the migration order. With 25 m*M* SBCD and 10 m*M* MBCD in the separation buffer, the histamine peak lay between phenylethylamine and tyramine, whereas at 25 m*M* SBCD and 5 m*M* MBCD, histamine migrated between cadavarine and phenylethylamine with an extended separation time of 45 min. A separation potential of +25 kV was optimal, since at higher potentials the resolution was adversely affected whereas at lower potentials the total migration time became very lengthy. Higher concentrations (75–100 m*M*) of borate in the running buffer also increased the total migration time. Ethanol was added to the separation buffer to enhance the solubility of the derivatives as well as the separation efficiency. The peak at 13.2 min shown in Fig. 2 was due to unreacted OPA. Fresh running buffer (150–200 μl) in both the inlet and outlet vials was used for each run. Otherwise, the migration times for the peaks were not as reproducible, possibly due to ethanol evaporation and/or the change in buffer ion concentration between the inlet and outlet vials. The detection limit ($S/N=3$) for the OPA derivatives using the UV detector was about 10 μ*M*. If the derivatized samples were left for 4 h at room temperature and at 5°C, the decrease of the integrated peak area for the derivatives ranged from 7 to 20% and 0 to 5%, respectively. These results corresponded well with those reported in the previous section for the fluorometric stability study.

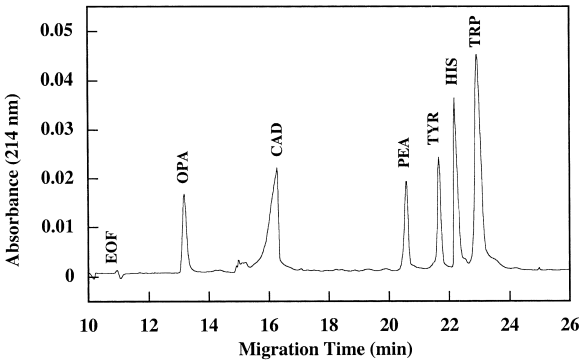

Fig. 2. Separation of five OPA derivatives (2 m*M* for cadavarine: CAD, histamine: HIS, tryptamine: TRP and 1 m*M* for tyramine: TYR and 2-phenylethylamine: PEA). The separation buffer consisted of 50 m*M* borate, pH 9.0, 20 m*M* MBCD, 25 m*M* SBCD and 10% ethanol (at 25°C, +25 kV, detection at 214 nm, with a resultant current of 70 μA).

3.3. Separation of OPA derivatives by CE using LIF detection

All eight biogenic amines were derivatized for studies using CE–LIF. Samples were run the same day as the derivatization reaction to maintain stability. The detection sensitivity was very dependent upon the resultant current during the electrophoretic run, especially in the case of the spermine and spermidine derivatives. For example, using the optimal conditions for UV detection the peaks for spermine and spermidine were hardly detectable as the current was 70 μA. However, the current could be significantly reduced by lowering the applied potential from 25 to 20 kV as well as the capillary temperature from 25 to 15°C. This lead to an increase in the detection limit by at least two- to threefold, likely due to an enhanced stability of the derivatives in the capillary. Lowering the borate buffer concentration from 50 to 15 m*M* further reduced the current, as well as reducing the total migration time. This change significantly enhanced the detection sensitivity of the spermine and spermidine derivatives. The separation resolution was further improved by reducing MBCD from 20 to 15 m*M*, resulting in a final current of only 40 μA (Fig. 3C). Unreacted OPA was consumed by glutamic acid to generated a major fluorescent peak at 37 min and a minor peak at 28 min, which did not interfere with the separation of the biogenic amine derivatives.

The addition of ethanol to the running buffer exhibited a pronounced effect on the separation of the OPA derivatives. Without ethanol in the separation buffer, the electropherogram displayed three problematic doublets: cadavarine/putrescine, phenylethylamine/tyramine, and histamine/tryptamine (figure not shown). As the concentration of ethanol increased to 5%, the doublets split more apart and finally baseline separation was achieved at a concentration of 10% ethanol. Acetonitrile was inferior as an organic modifier for separation as the baseline was less stable. The effect of the MBCD to SBCD concentration ratio was observed by holding the level of MBCD constant at 15 m*M* and increasing SBCD from 15 m*M* (Fig. 3A) to 20 m*M* (Fig. 3B) and finally to 25 m*M* (Fig. 3C) where baseline resolution was achieved.

The importance of the removal of residual OPA by the addition of glutamic acid shortly after (1 min) the reaction with the biogenic amines was clearly demonstrated in Fig. 4. With respect to detection of the OPA derivatives (0.5 μ*M*) around the detection limit, the entire electropherogram was masked by the residual OPA if the glutamic acid step was omitted in

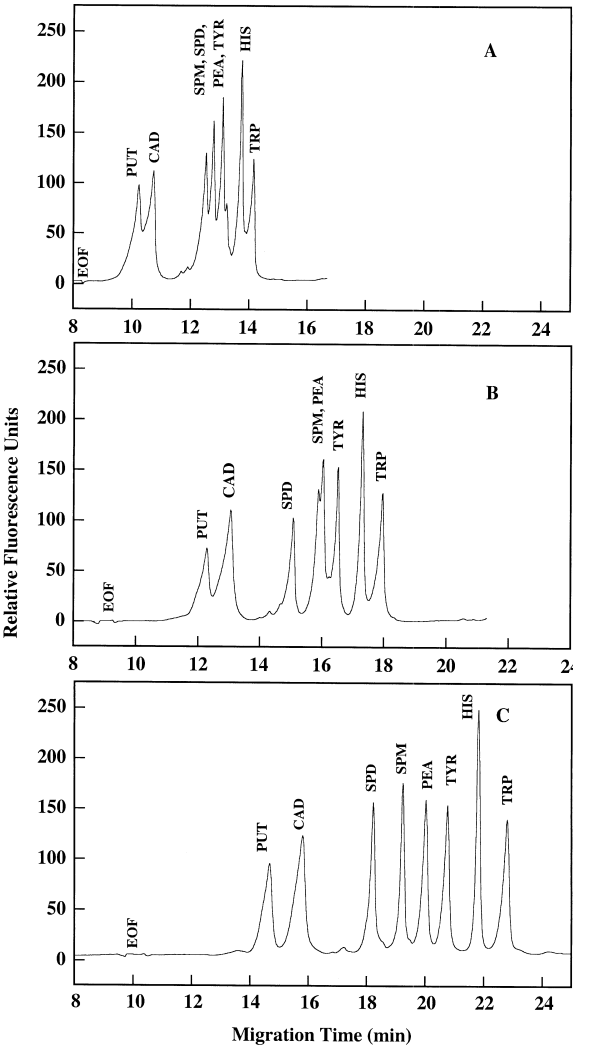

Fig. 3. Effect of cyclodextrin ratio on the CE–LIF separation of OPA derivatives (50 μ*M*) at 15°C and +20 kV. The separation buffer contained 15 m*M* borate, pH 9.0, 10% ethanol, 15 m*M* MBCD, and 25 m*M* SBCD. Abbreviations: putrescine, PUT; spermine, SPM; spermidine, SPD and all others are the same as in Fig. 2. (A) 15 m*M* MBCD–15 m*M* SBCD: 29 μA; (B) 15 m*M* MBCD–20 m*M* SBCD: 35 μA; (C) 15 m*M* MBCD–25 m*M* SBCD: 40 μA.

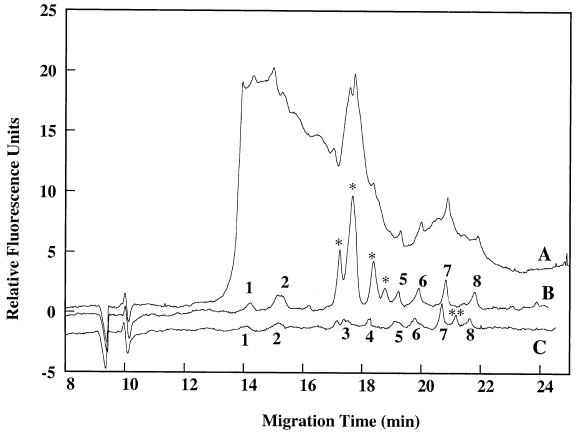

Fig. 4. Effect of glutamic acid (GLU) on the derivatization reaction to remove residual OPA. The concentration of OPA derivatives was 0.5 μ*M* and other conditions were the same as in Fig. 3C. (A) 4 m*M* OPA: 0 GLU; (B) 4 m*M* OPA: 10 m*M* GLU; peaks: 1=PUT, 2=CAD, 3=SPD (under *), 4=SPM (under *), 5=PEA, 6=TYR, 7=HIS, and 8=TRP; (C) 0.1 m*M* OPA: 10 m*M* GLU. Peak identification as in (B), **=unidentified peak.

the derivatization protocol (electropherogram A). In the presence of 10 m*M* glutamic acid (electropherogram B), the resolution was greatly improved and only the peaks for the spermine and spermidine derivatives were masked. However, the detection of spermine and spermidine around the detection limit could still be attained by lowering OPA from 4 to 0.1 m*M* (electropherogram C). This was of importance for analysis of spermine and spermidine in which samples can neither be preconcentrated nor contain significant amounts of other amines.

Reproducibility of the separation, under the operating conditions of Fig. 3C, was very good as shown in Table 1. The reproducibility of the migration times ($n=8$) for the 8 OPA derivatives was within 0.5% at a 95% confidence interval. For this experiment the same stock sample stored on ice containing all eight derivatives was run eight times consecutively, using a new aliquot for each run. The reproducibility for the integrated area ($n=17$) of the eight OPA derivatives (50 μ*M*) ranged from 3 to 8% at a 95% confidence interval. This experiment was carried out over 2 days using two different stock samples, stored on ice throughout the experiment. The stability at this concentration was excellent since the derivatized samples left at 5°C during the course of this experiment exhibited the same integrated areas for all OPA derivatives. Such a result was anticipated from the fluorometry studies as mentioned earlier. Standard calibrations were obtained for each of the eight OPA derivatives using six different concentrations (100, 50, 25, 10, 2.5, and 1 μ*M*) in a mixture of all derivatives. At a 95% confidence interval the error on the sensitivity ranged from 1 to 7%. The separation was considered to be very satisfactory under these operating conditions since the resolution (R_s, $n=17$) of all peaks was greater than 1.5. The separation efficiency (N/m, $n=17$) was reasonable with respect to the bulky structure of the derivatives, as all analytes resulted in peaks with theoretical plates/m between $2\cdot10^4$ and $2\cdot10^5$. The number of theoretical plates obtained in this study is similar to those previously reported for bulky polyaromatic hydrocarbons [32] and resin acid derivatives [33], which also used mixtures of SBCD and MBCD for LIF electrophoretic separations. The

Table 1
Separation of eight OPA derivatives by CE–LIF with respect to migration time, reproducibility, sensitivity, resolution and separation efficiency (N/m)

OPA derivative	Migration time (min)	Area[a] (in million)	Sensitivity (in million/μ*M*)	Resolution (R_s)	Efficiency (N/m)
Putrescine	14.78±0.06	2000±100	40.8±1.2	–	19 800
Cadavarine	15.96±0.06	3200±160	62.5±1.4	1.57	17 400
Spermine	18.25±0.06	2400±74	41.8±1.7	1.72	90 300
Spermidine	19.19±0.08	1800±133	33.0±2.2	2.30	99 200
2-Phenylethylamine	20.11±0.09	2200±86	42.0±0.64	2.43	109 000
Tyramine	20.87±0.09	2100±80	42.1±0.46	1.88	125 000
Histamine	21.83±0.11	2400±83	46.2±0.72	2.83	187 000
Tryptamine	22.91±0.11	2200±99	42.4±0.47	2.60	107 000

[a] Obtained using 50 μ*M* of the derivatized samples.

detection limit ($S/N=3$) was determined to be 250 n*M* (with the exception of spermine and spermidine) a significant improvement of 40-fold compared to UV detection. The detection limits for spermine and spermidine were 2.5 and 10 μ*M*, respectively, but as previously mentioned this could be improved by lowering the concentration of OPA. Using the extended light path capillary for LIF detection the sensitivity of the OPA derivatives was increased about threefold for each derivative, achieving a detection limit of about 100 n*M*. However, it should be noted that the resolution using the extended light path capillary was slightly hindered in comparison, likely due to analyte dispersion at the detector window.

3.4. Detection of biogenic amines in samples

The accumulation of biogenic amines over time at different storage temperatures was detected in both rainbow trout and sole using CE–LIF. After 4 days at 5°C detectable levels of both putrescine and cadavarine could be observed in both rainbow trout and sole (Fig. 5A and B). Histamine was detected in trout but not sole as previously reported by Male et al. [29]. Trace amounts of phenylethylamine and tyramine were also detected, however, there was no significant accumulation of these two amines during storage. As expected, the levels of these fish spoilage markers increased with storage time as well as storage temperature for both rainbow trout and sole (figure not shown), as determined by CE–LIF. The regulatory action level for most fish in Canada is >10 mg histamine/100 g of flesh [34] (or about 0.30 m*M* in this study). In this experiment, the trout sample reached this threshold level within 4–5 days at 5°C or 1–2 days at room temperature. The reproducibility was determined for eight repeated analyses using a trout samples left at 5°C for 7 days. At a 95% confidence interval the error was 7, 13, and 17% for putrescine (83±6 μ*M*), cadavarine (340±44 μ*M*) and histamine (861±146 μ*M*), respectively. The values obtained for cadavarine and putrescine using CE–LIF compared very well with those of the spectrophotometric assay. The enzymatic values versus the CE values resulted in straight lines with slopes of 1.00±0.03 and 1.04±0.06 with correlation coefficients of 0.997 and 0.981 (95% confidence interval, $n=14$) for putrescine and cadavarine, respectively (figure not shown).

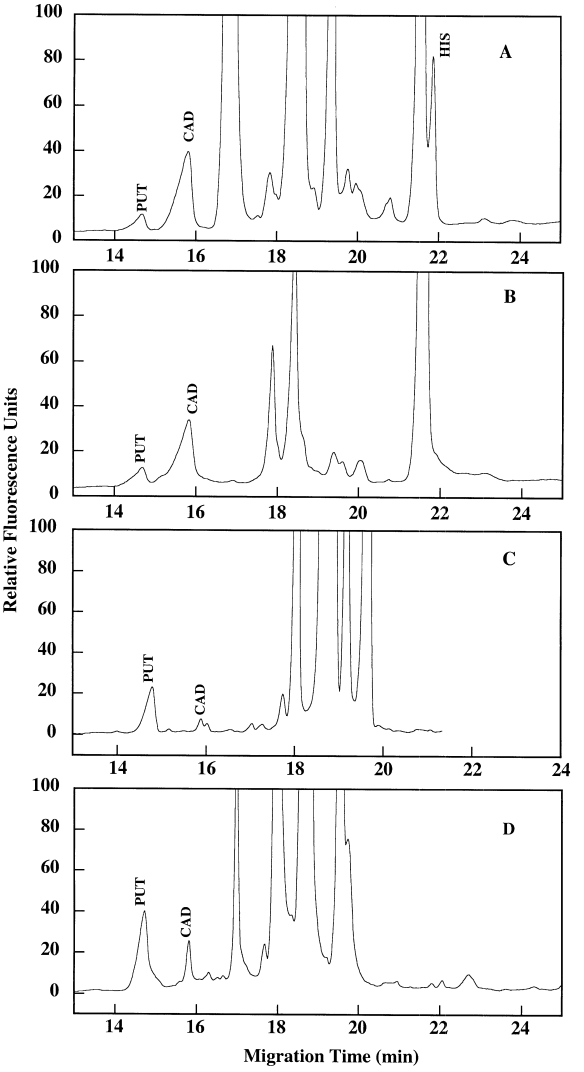

Fig. 5. CE–LIF detection of biogenic amines. Separation conditions were the same as in Fig. 3C. (A) Rainbow trout at 5°C for 7 days, (B) sole at 5°C for 7 days, (C) white wine and (D) red wine.

Both white and red wines were detected to contain noticeable levels of putrescine and cadavarine (Fig. 5C, D). Putrescine levels were higher at 45 and 84 μ*M* compared to cadavarine levels at 6.4 and 11 μ*M* for the white and red wines, respectively. The CE values could not be verified by the spectrophotometric assay since some components in the wines inhibited the enzyme assay. However, the concen-

trations reported here are similar to the levels reported for other white and red wines [13–15]. A hydrolyzed urine sample was also derivatized by OPA and tested by CE–LIF for the presence of putrescine, a marker known to be elevated in urine of cancer patients [11,30] Trace amounts of putrescine were detected (figure not shown) after urine hydrolysis of acetylputrescine to putrescine. Although the value of putrescine could not be verified by the enzymatic assay since the amount of urine required to detect such a low level inhibited the enzymatic activity, this level (10.1 μM) was similar to the literature data reported for healthy subjects. It would be anticipated that peaks for both putrescine and cadavarine would become more pronounced in the case of cancer patients where the total level of polyamines is normally greater than 30 μM [30].

4. Conclusion

This study has demonstrated the stabilization of OPA derivatives of biogenic amines by forming complexes with MBCD. This was a significant development, since it enables sample preparation and storage in advance, thus eliminating tedious pre- and post-column derivatization methods currently employed in HPLC. The interference caused by residual OPA was eliminated by adding glutamic acid after the initial derivatization step. For CE–LIF, on-line derivatization becomes even more complicated and cumbersome, therefore the enhanced stability lends itself well to this powerful technique. With cyclodextrin-modified CE, putrescine and cadavarine were detected in both red and white wine samples as well as in rainbow trout and sole during the spoilage process.

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