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Analysis of biogenic amines by solid-phase microextraction and high-performance liquid chromatography with electrochemical detection

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Abstract

We investigated the new solid-phase microextraction method by high-performance liquid chromatography with electrochemical detection for the analysis of biogenic amines. The Carbowax–Templated Resin 50 μm (purple) fibre coating offers good performances for dopamine and serotonin separation, i.e., good selectivity and high sensibility (0.1 $\mu g\ l^{-1}$). We also tested this fibre for biogenic amines quantification of rat striatum. The coating seems to be selective towards the amines and has low affinity for the metabolites, allowing a good separation and preventing drawbacks from the biological matrix. These first results obtained using this original separation method offer large perspectives of application to many biological samples.

Keywords: Extraction methods; Solid-phase microextraction; Biogenic amines; Dopamine; Serotonin

1. Introduction

Biogenic amines (mainly dopamine, serotonin, norepinephrine and octopamine) are molecules of paramount importance in the whole animal kingdom. These substances, present in the central nervous system (CNS) of various species, are very much conserved through evolution. They are implicated in the regulation of a large range of behavioural and physiological systems. For instance, in invertebrates, they regulate movement and locomotion in the cockroach [1] and in crickets [2], and they modulate social interactions in crustaceans [3] and ants [4]. Biogenic amines also participate in learning processes in honeybees [5]. In higher vertebrates and humans, biogenic amines are involved in the regulation of sleep [6], mood [7] and emotions [8]. They, therefore, constitute the subject of numerous studies in the field of neuroscience. However, their quantification in the CNS, using high-performance liquid chromatography (HPLC) connected with electrochemical detection (ED), currently suffers important drawbacks. A frequent problem comes from a lack of good separation between monoamines themselves and their metabolites commonly abundant in biological samples.

The solid-phase microextraction (SPME) sampling technique based on adsorption is currently used to separate analytes from sample matrix [9]. This technique offers several advantages in analysing biological samples. It is a non destructive method which allows multiple use of one vial. Furthermore, SPME fibres present interesting selective adsorption properties. A new interface was developed by Supelco (Bellefonte, PA, USA) to hyphenate SPME

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with HPLC. Such a device has given good results both in quantitative and qualitative analysis [10–12]. We tested this commercial interface for biogenic amines analysis with HPLC–ED. We tried different available fibre coatings on biogenic amine and metabolite standard mixtures as well as on rat brain striatum extracts.

2. Materials and methods

2.1. Reagents

3-Hydroxytyramine (dopamine, DA), 5-hydroxytryptamine (serotonin, 5-HT), 3,4-dihydroxyphenylacetic acid (dopac), 5-hydroxyindolacetic acid (5HIAA), 1-octanesulphonic acid (OSA), homovanilic acid (HVA), methanol (MeOH), perchloric acid, $Na_2S_2O_5$ and EDTA were obtained from Sigma (St. Louis, MO, USA) and Aldrich (Milwaukee, WI, USA). Citric acid and sodium acetate were obtained from Prolabo.

2.2. General procedure

The fibre was plunged into a 4-ml vial containing the extract. Firstly, we investigated the properties of four available SPME fibres in a DA/5-HT aqueous solution (10 $\mu g\ l^{-1}$). Peak areas were compared to those obtained with classical injection of 50 μl of the same solution. Thus, we could estimate the percentage of the total DA and 5-HT amounts adsorbed on the fibre and desorbed in the chamber. Thereafter we only used the fibre which offered the best results.

In a second experiment, we investigated the influence of the adsorption duration. We plunged the fibre in the same DA/5-HT solution (10 $\mu g\ l^{-1}$) for 5, 15, 30, 60 and 120 min.

In a third experiment we analysed the effect of the desorption duration by testing 5, 10 and 15 min (DA/5-HT, 10 $\mu g\ l^{-1}$ in the mobile phase described below).

We also analysed the effect of the standard DA and 5-HT sample concentration by testing solution of 0.05, 0.1, 0.2, 0.5, 1 and 10 $\mu g\ l^{-1}$.

Finally, we analysed a biological sample (rat striatum) both with a classical injection protocol (50 μl) and with SPME (in a 4-ml vial). Biological sample chromatograms were compared to those obtained with a standard solution composed of DA, 5-HT, 5-HIAA, dopac and HVA (10 $\mu g\ l^{-1}$).

2.3. Solid-phase microextraction

The SPME device is a modified syringe. The plunger moves a fibre in and out of a metallic needle which protects the fibre coating. In our experiments, we tested the properties of four fibre coatings: Carbowax–Templated Resin 50 μm (CW–TPR, purple fibre); polydimethylsiloxane–divinylbenzene 65 μm (PDMS–DVB, brown fibre); polydimethylsiloxane 60 μm (PDMS, red fibre) and polyacrylate 85 μm (white fibre)

These coatings provide different adsorption properties for different kinds of analytes.

The SPME interface consists of a desorption chamber (200 μl) placed instead of the injection loop connected to a classical injection valve.

2.4. Biogenic amine separation and electrochemical detection

Biogenic amines were separated using a C_8+ Upchurch HPLC column (25×4.6 mm, 5 μm particles) according to an adapted procedure previously described [13]. The mobile phase contained citric acid (0.1 *M*), sodium acetate (0.1 *M*), OSA (400 mg) and MeOH (15%). pH was adjusted to 5.5. The mobile phase, filtered through a 0.45-μm filter (Millipore) and degassed, was pumped at 0.5 ml min^{-1} with a Bio-Rad HPLC pump.

Biogenic amines were detected with an Antec electrochemical detector equipped with an amperometric electrode set at an oxidising potential of 780 mV. Data were processed using the Millenium software (v2.15) from Waters for personal computers.

2.5. Biological sample preparation

Male Wistar rats weighing 250–300 g were killed by decapitation and brains were immediately dissected on ice. Tissues (striatum) were homogenised with an Ultra Turax T25 (Bioblock Scientific, Vernon Hills, IL, USA) in 0.5 ml of perchloric acid (0.2 *M*), EDTA (1 g l^{-1}) and $Na_2S_2O_5$ (1 g l^{-1}). Tissues

Table 1
Peak areas (median values, $n=3$) and respective quantities of dopamine and 5-HT measured with classical injection protocol and using different SPME fibres (DA and 5-HT solutions, 10 μg l^{-1})[a]

	Classical injection (50 μl)	Purple fibre (CW–TPR)	Brown fibre (PDMS–DVB)	Red fibre (PDMS)	White fibre (polyacrylate)
DA peak area (μV s)	1 767 435	582 537	23 110	31 979	Undetectable
DA quantity (pg)	500	165	6	9	–
5-HT peak area (μV s)	2 563 874	2 286 683	65 321	15 131	Undetectable
5-HT quantity (pg)	500	446	13	3	–

[a] Best results were obtained with the purple fibre which allowed the detection of 165 pg of DA and 446 pg of 5-HT which represent 0.41% and 1.15% of respective quantities in the 4-ml vial.

were then centrifuged at 7310 *g* (Beckman J2-21 M/E centrifuge; JA 21 rotor, Beckman Instruments, Fullerton, CA, USA) for 10 min at 4°C. Supernatants were kept at −80°C until use.

Finally, 4 ml of water was added to samples just before the fibre was plunged in. Control consisted of a 50 μl classical injection of the same 4.5 ml sample.

3. Results and discussion

Table 1 presents comparative results obtained with classical injection of the sample and SPME injection using four different fibres. The purple fibre (CW–TPR) offered best performances. Fifteen min adsorption and 5 min desorption DA and 5-HT resulted in 582 mV s^{-1} and 2287 mV s^{-1} peaks, respectively. These areas corresponded to 165 pg (DA) and 446 pg (5-HT) (calculated from the classical injection of the external standard) and represented 0.41% and 1.12% of the total DA and 5-HT quantity in the 4-ml vial (10 μg l^{-1}). DA and 5-HT did not have the same behaviour. With a classical injection DA gave larger peaks than 5-HT. On the contrary, with SPME, 5-HT gave larger peaks than DA.

We investigated the effect of the adsorption duration with the purple fibre (Fig. 1). The maximum peak areas were reached after 30 min for DA and

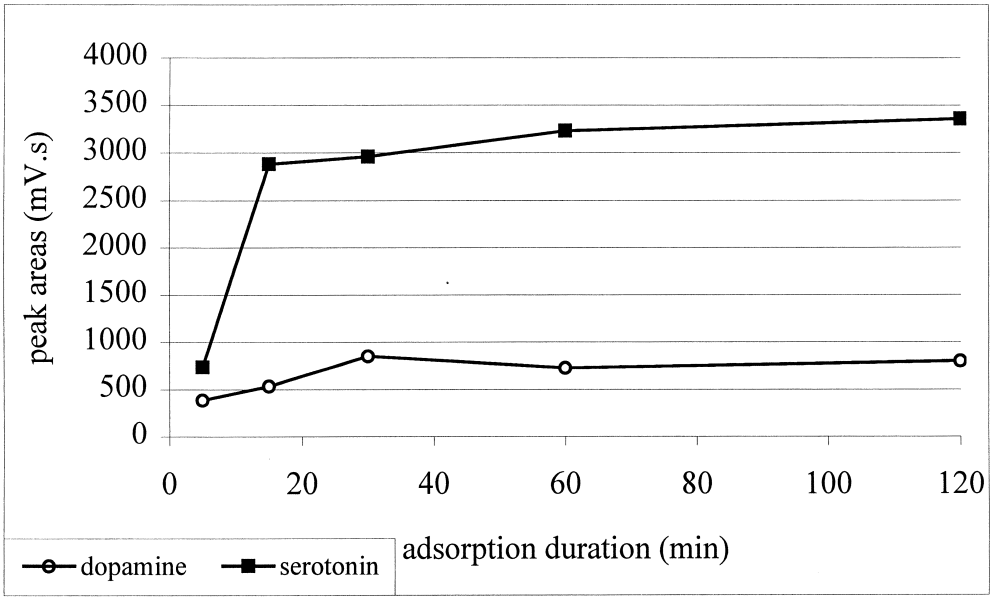

Fig. 1. Influence of the duration of adsorption. Values are median ($n=3$).

after 120 min for 5-HT. A 15-min adsorption duration allowed the transfer of about 85% of this maximum quantity. We therefore used a 15-min adsorption duration in all further experiments.

Fig. 2 presents the desorption duration effect. A 5-min long desorption duration (with 15 min adsorption) did not allow the complete desorption of the compounds adsorbed on the fibre. However, a 10-min long desorption offered only a gain of 21% for DA and 8% for 5-HT. We therefore conducted all further experiments with a 5-min long desorption.

We also tested the purple fibre with DA and 5-HT standards of different concentrations, from 0.1 $\mu g\ l^{-1}$ to 10 $\mu g\ l^{-1}$, with 15 min adsorption and 5 min desorption. We obtained a detection limit around 0.1 $\mu g\ l^{-1}$ and we found good correlations ($r=$ 0.9951 and $r=0.9998$) between concentrations and respective DA and 5-HT peak areas.

Fig. 3a shows the chromatogram (two amines plus three acids) obtained with classical injection (10 $\mu g\ l^{-1}$; 50 μl). The five compounds gave large, sharp and well separated peaks. A chromatogram obtained with the same standard solution but using SPME is shown in Fig. 3b. In this case, amine peaks are still large, but acids (dopac, 5-HIAA and HVA) are present only as traces. Thus, we suppose acids are to be adsorbed with a lower affinity.

Fig. 4a shows results obtained with a rat striatum extract. DA, 5-HT, dopac, 5-HIAA and HVA, however, many other peaks are also present. Such peaks comes from the biological matrix which contains many compounds (3-methoxytyramine, tryptophan, tyramine), etc. Moreover the dopamine peak is not completely separated from the precedent peak. The SPME injection gave peaks of lower area as compared to the peaks obtained after classical injection (Fig. 4b). Those peaks are well separated especially the one of dopamine. Indeed, SPME technique avoid large peaks due to the matrix substances. The comparison in SPME injection of pure standards with biological sample shows that the presence of interfering compounds reduced the transfer of metabolites.

Thus, the SPME technique seems to offer good results for biogenic amine detection with HPLC–ED even with low concentrations. It allows a good separation between amines and acids and it reduces drawbacks due to the biological matrix. The results presented here offer large suitable applications to different biological samples. Especially, we propose the use of this technique when samples with very high metabolite/amine rapport are used. Thus, this method would be of greater interest of use for biological samples such as plasma and urine, which

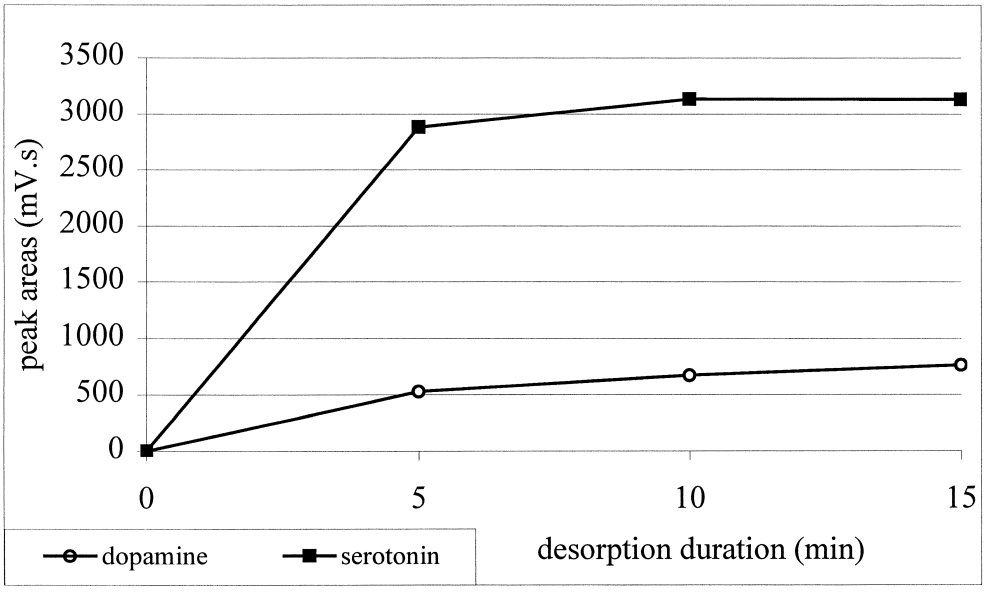

Fig. 2. Influence of the desorption duration. Values are median ($n=3$).

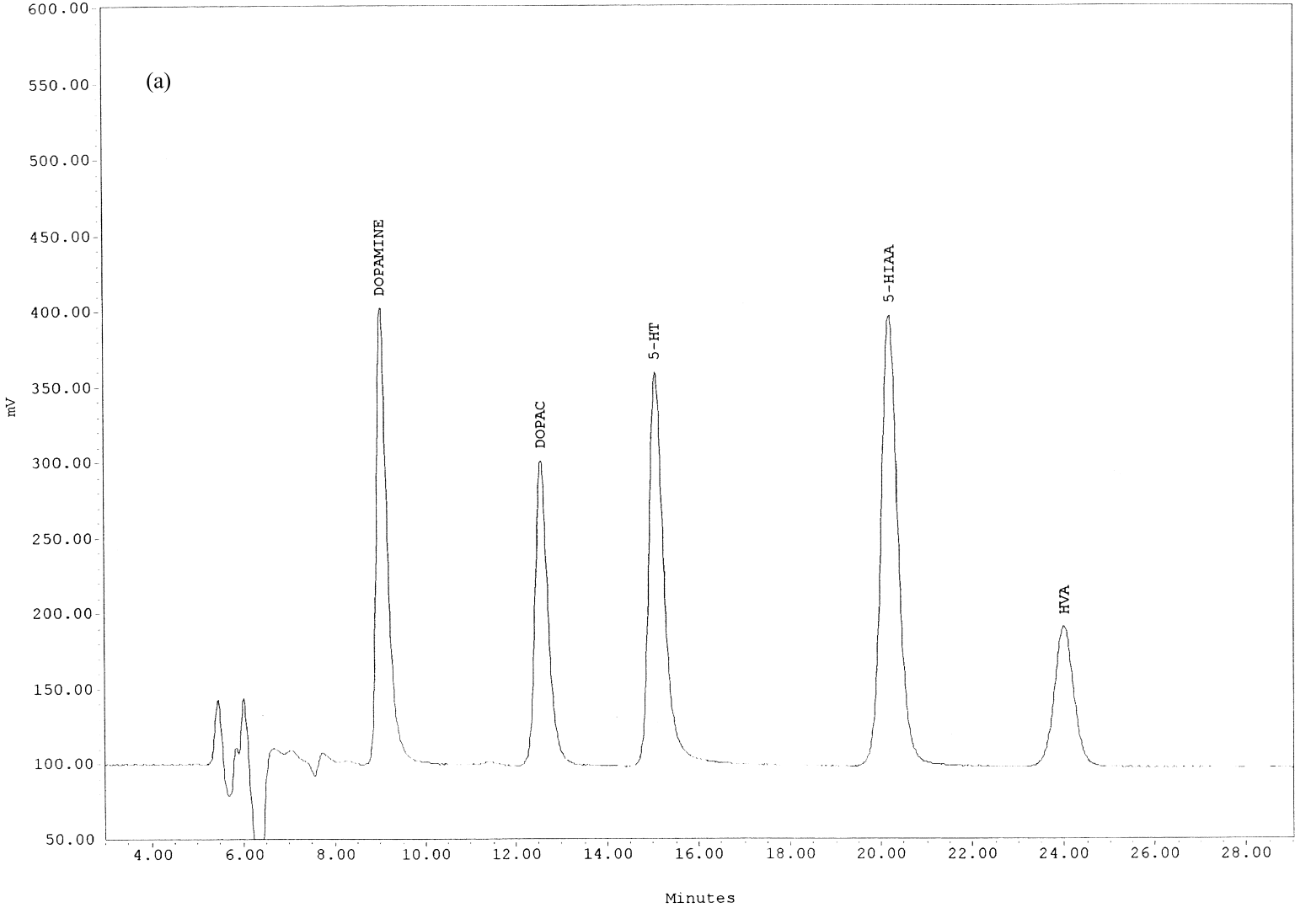

Fig. 3. Chromatograms of standard sample obtained with classical injection (a) and with SPME (b).

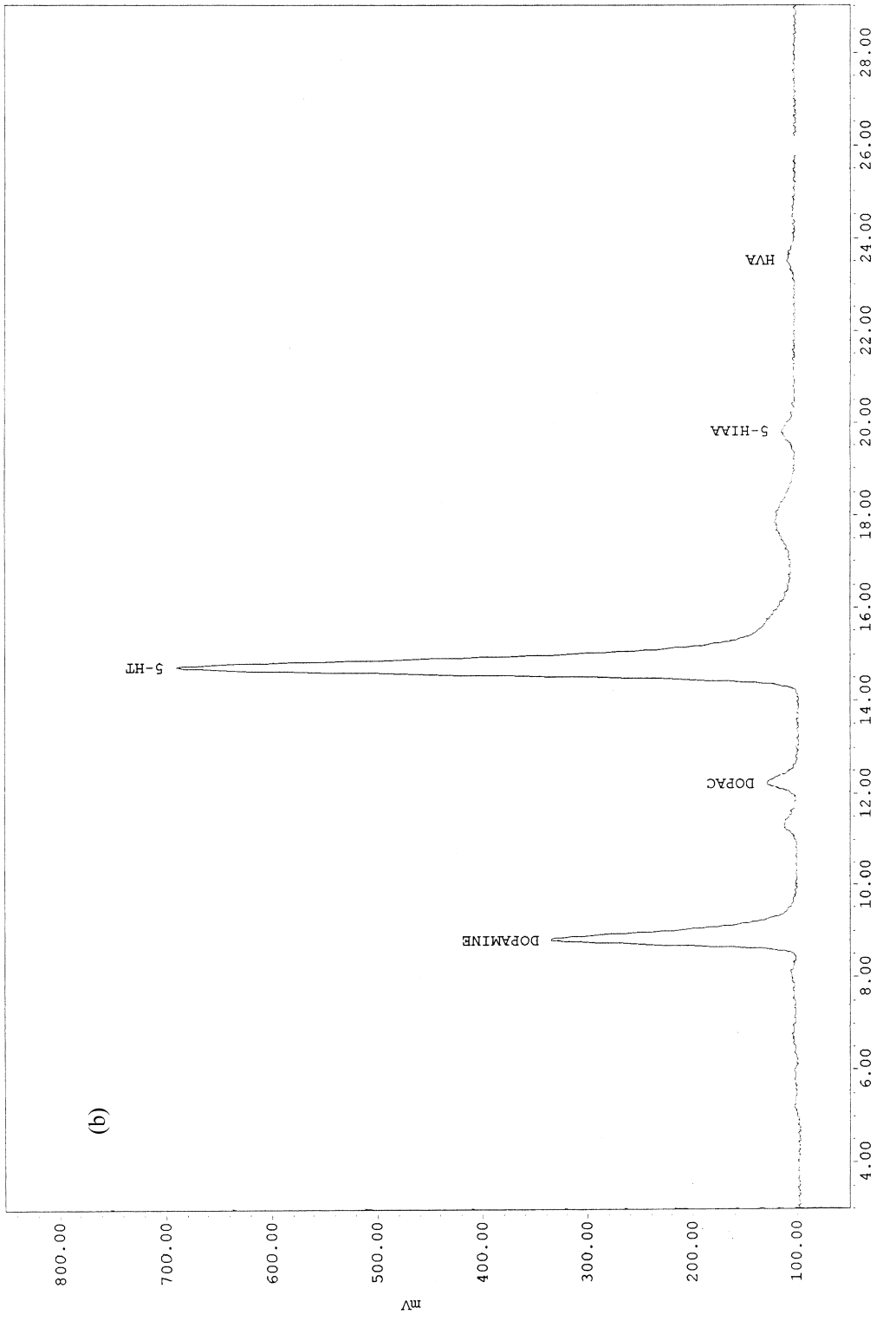

Fig. 3 (*continued*).

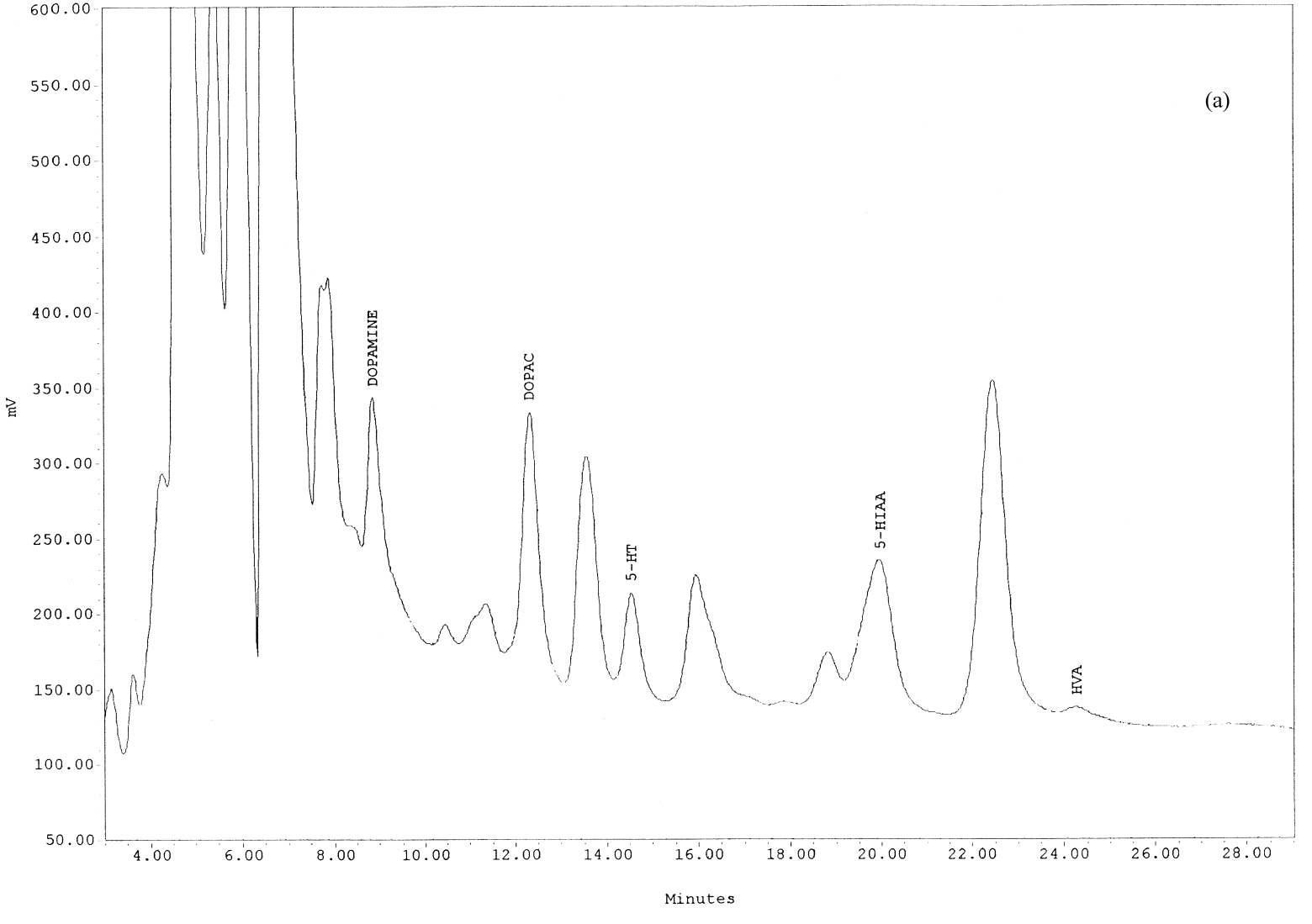

Fig. 4. Chromatograms of a rat stratum sample obtained with classical injection (a) and with SPME (b).

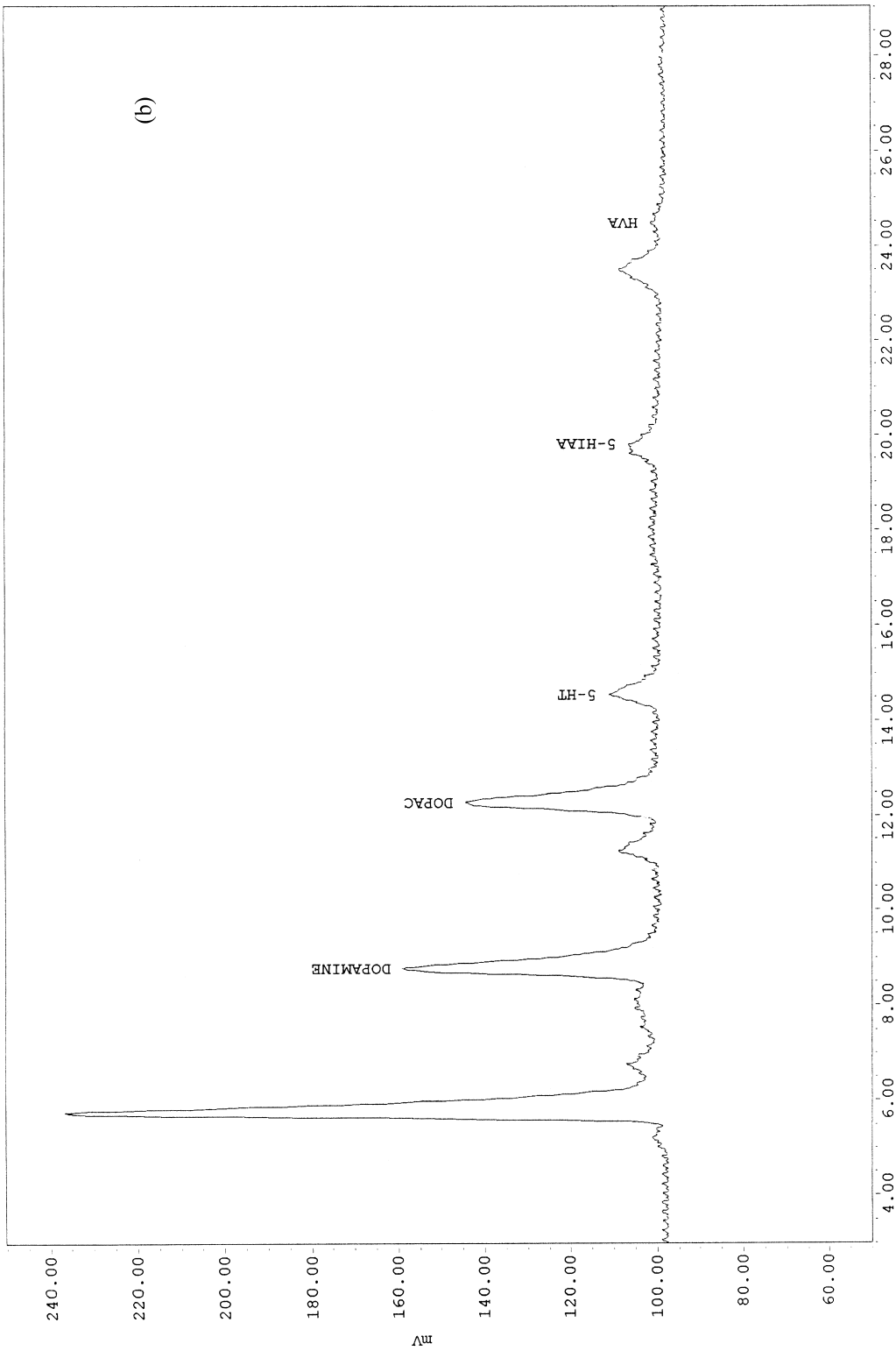

Fig. 4 (*continued*).

often require complex phases of extraction. However, technical specificities should be determined according to each biological matrix of interest, regarding to the range of concentration of amines present.

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