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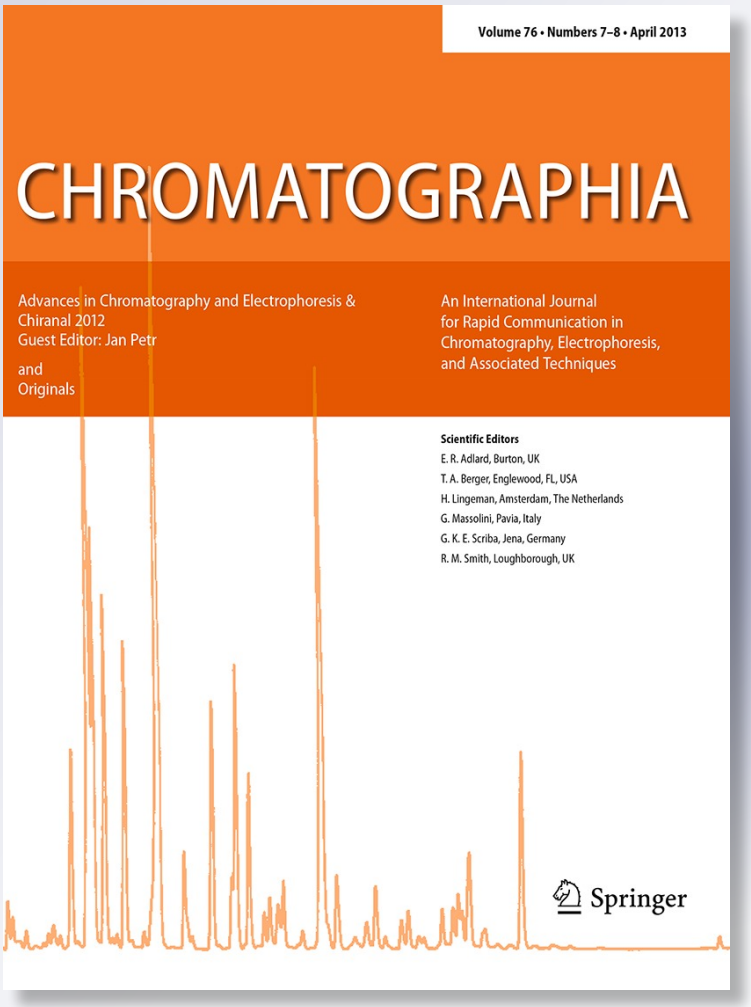

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ORIGINAL

Spectrometric and Chromatographic Study of Reactive Oxidants Hypochlorous and Hypobromous Acids and Their Interactions with Taurine

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Abstract In this study, we focused on the studying of taurine complexes with phenol and sodium hypochlorite, and of taurine with sodium hypobromite by spectrometry, reverse phase chromatography and ion-exchange chromatography. The formed complexes were studied under various conditions such as temperature (10, 20, 30, 40, 50 and 60 °C), and/or time of interaction (0, 5, 10, 15, 20, 25 and 30 min). In addition, we optimized high performance liquid chromatography coupled with UV detector for detection of taurine and its complexes with the acids. Taurine–phenol–hypochlorite complex was effectively separated under isocratic elution, mobile phase water:methanol 30:70 %, *v*:*v*, flow rate 1 mL min^{-1} and 55 °C. Taurine-bromamine complex was isolated under the following optimized conditions as isocratic elution, mobile phase water:methanol 85:15 % *v*:*v*, flow rate 1 mL min^{-1} and 55 °C. The limits of detection (3 S/N) were estimated as 1 μM for both types of complexes, i.e. for taurine. Further, we estimated recovery in one sample of urine (male 25 years), commercially achieved energy drink and tea leaves and varied from 79 to 86 %. Further, we aimed our attention at investigating the ability of the above characterized taurine and taurine complexes to scavenge reactive oxygen species. For this purpose, an ion-exchange liquid chromatography with post-column derivatization with ninhydrin and VIS detector was used. It clearly follows from the results obtained that taurine itself reacts with peroxide more intensely than in a bound form, which can be associated with the highest signal decrease. Complexes stabilized structure taurine against peroxide radicals, resulting in slower decreasing of peak heights. The most stable was taurine complexes with phenol and hypobromite.

Keywords Reversed phase HPLC · Ion-exchange chromatography · Spectrophotometry · Complexes · Taurine · Antioxidant

Introduction

Activated neutrophils and eosinophils generate a variety of reactive oxygen species (ROS). Hypochlorous acid (HOCl) and hypobromous acid (HOBr) are the major reactive oxidants generated by these cells at sites of inflammation [1]. Both agents, components of the human innate immune system, exert strong antimicrobial activity, but their excessive production leads to tissue damaging [2, 3]. Taurine is the most abundant free amino acid in the leukocyte cytosol (30 mM) and is the major scavenger for both hypohalous acids, HOCl and HOBr [4]. Mammalian taurine (2-aminoethanesulfonic acid) is synthesised in the pancreas via the cysteine sulfinic acid pathway [5]. In this pathway (Fig. 1), the sulfhydryl group of cysteine is first oxidized to cysteine sulfinic acid by the enzyme cysteine dioxygenase. Cysteine sulfinic acid, in turn, is decarboxylated by sulfinoalanine decarboxylase to form hypotaurine [6]. Moreover, taurine has significant anti-inflammatory

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L. Nejdl · J. Sochor · O. Zitka · B. Ruttkay-Nedecky · P. Kopel · V. Adam · J. Hubalek · R. Kizek (✉)
Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic
e-mail: kizek@sci.muni.cz

J. Sochor · O. Zitka · N. Cernei · B. Ruttkay-Nedecky · P. Kopel · P. Babula · V. Adam · J. Hubalek · R. Kizek
Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, 616 00 Brno, Czech Republic

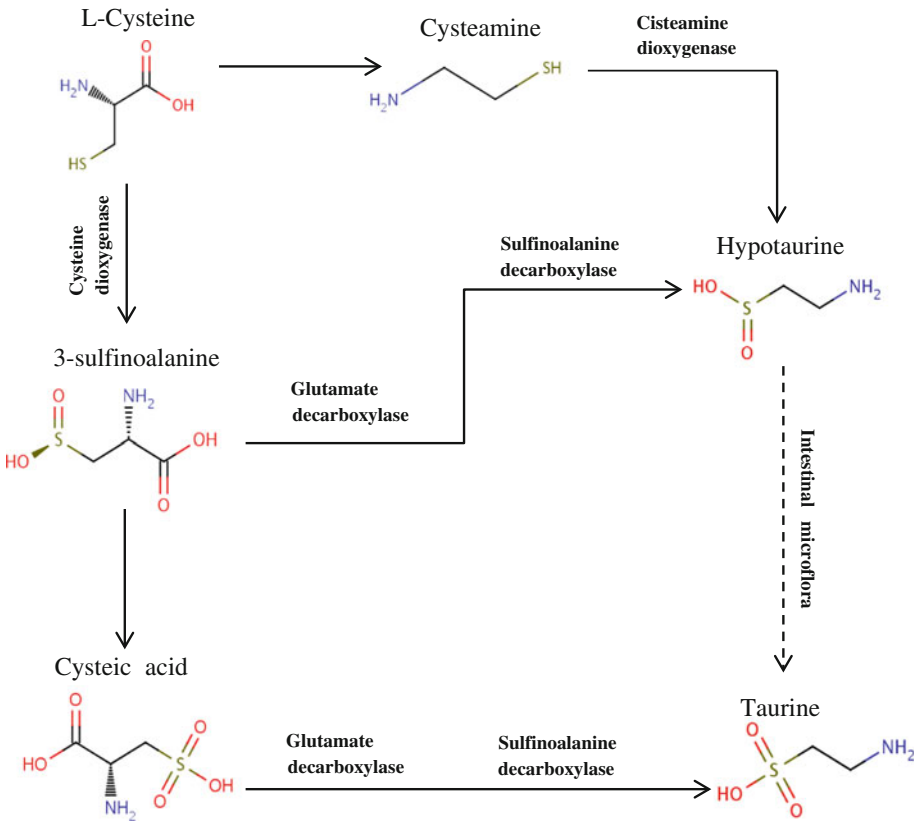

Fig. 1 Taurine and hypotaurine metabolism in *Homo sapiens,* modified in accordance with Koplove et al. [48]

properties [7] and participates in different physiological processes as it stabilizes cell membranes [8], regulates fatty tissues metabolism [9] and levels of calcium ions in blood [10]. Due to biological functions of taurine, this compound is intensely studied due to its possibility to regulate oxidation stress [1, 6, 11–15]. Protective role of taurine against cadmium-induced oxidative stress in murine erythrocytes was demonstrated [16–18]. Treatment with taurine before cadmium intoxication prevented the toxin-induced oxidative impairments in the erythrocytes of the experimental animals [16].

Derivatization with subsequent high performance liquid chromatographic (HPLC) [19–28] separation is the most commonly used techniques for taurine quantification. *o*-Phthaldialdehyde (OPA) [29], 2,4-dinitrofluorobenzene (DNFB) [30], 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl-Cl) [31], and 4-dimethylaminoazobenzene-4′-sulfonyl chloride (DABS-Cl) [32] are probably the most commonly used derivatization agents [33, 34]. Hyphenated techniques, such as HPLC Fourier transform infrared (spectroscopy) and HPLC inductively coupled plasma atomic emission spectroscopy, have been proved to be effective for the analysis of taurine in biological samples. However, they have not been widely used due to the complexity of the instruments [35]. Due to ability of taurine to form complexes with ninhydrin [36–39], *o*-phthalaldehyde [40] and l-fluoro-2,4-dinitrobenzene [41], it is possible to detect it also spectrophotometrically. Spectrophotometric measurements are usually cost effective and easy to perform. This fact is important especially in the light of the fact that biological significance of taurine derivatives remains almost unknown. It has been demonstrated that haloamines play a role in the inflammatory processes as proinflammatory mediators [1]. In addition, taurine halocomplexes show also antimicrobial properties.

The main aim of this study was to characterize complexes of taurine with hypochlorous and hypobromous acids using spectrometry (Fig. 2a, b). Primarily, we focused on the basic characteristics of the studied complexes using UV–vis spectrometry. Further, the characterized complexes were analysed using high performance liquid chromatography with UV detection.

Experimental Section

Chemicals

Taurine, phenol, sodium hypochlorite solution (10–15 %), sodium bromide, sodium phosphate tribasic dodecahydrate, sodium hydroxide, hydrochloric acid were purchased from Sigma Aldrich Chemical Corp. (St. Louis, MO, USA),

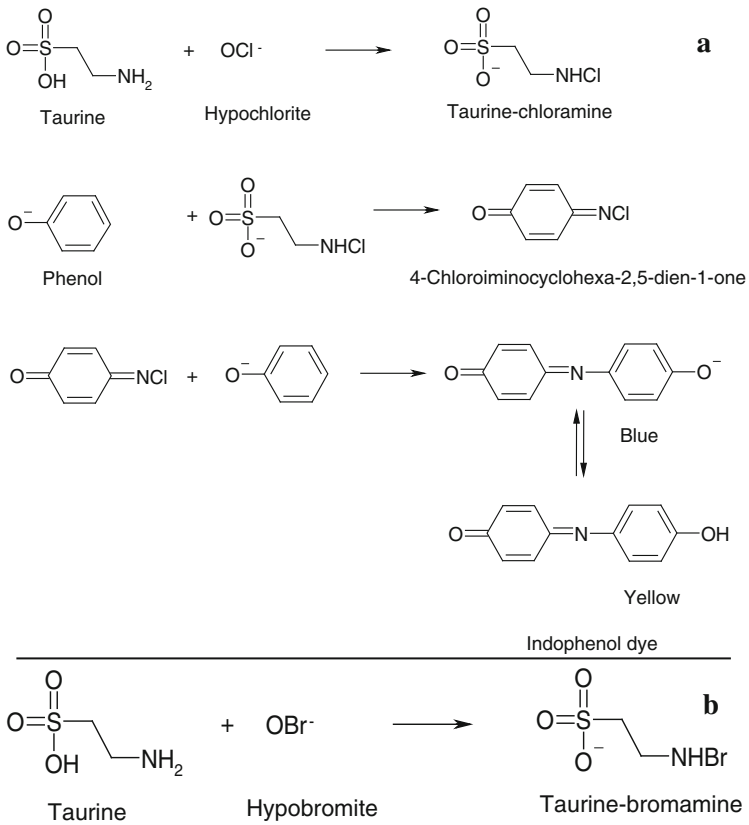

Fig. 2 **a** Complexes formed in reaction between taurine, phenol and sodium hypochlorite. **b** Complexes formed in reaction between taurine and sodium hypobromite

unless noted otherwise. The following stock solutions of the chemicals were prepared 800 mM taurine, 680 mM phenol, 300 mM NaClO by dilution with MilliQ distilled water. 100 mM NaBrO was prepared by mixing 100 mM NaBr with 100 mM HOCl (prepared from 100 mM NaOCl by adjusting to pH 4 with HCl).

Preparation of Taurine solutions

To study taurine complexes, taurine of the following concentrations was prepared: (a) 100, (b) 50, (c) 25, (d) 12.5, (e) 6.25, (f) 3.13 and (g) 1.56 mM. To study the influence of time and temperature on the measured absorbance, taurine of the following concentrations were prepared: (a) 4, (b) 2, (c) 1, (d) 0.5, (e) 0.25 and (f) 0.125 mM. MilliQ water was used for solution preparation.

Preparation of Complex of Taurine with Phenol and Sodium Hypochlorite

To micro-test tubes, the following chemicals were pipetted: 100 µL taurine 400 mM, 100 µL phosphate buffer 20 mM (pH 10.35), 100 µL phenol 680 mM and 100 µL sodium hypochlorite 300 mM [8]. MilliQ water was used for solution preparation. Time of interaction was 24 h.

Preparation of Complex of Taurine with Sodium Hypobromite

To micro-test tubes, the following chemicals were pipetted: 200 µL taurine 400 mM and 200 µL sodium hypobromite 100 mM [1]. MilliQ water was used for solution preparation. Formation of complex was monitored immediately after sample preparation.

Spectrophotometric Measurements

Spectra were recorded within the range from 220 to 800 nm on spectrophotometer SPECORD 210 (Analytik Jena, Jena, Germany) using quartz cuvettes (1 cm, Hellma, Essex, UK). Formation of complexes was monitored at periods of 0, 5, 10, 15, 20, 25 and 30 min. Cuvette space was tempered by a thermostat Julabo (Labortechnik, Wasserburg, Germany) to temperatures 10, 20, 30, 40, 50 and 60 °C.

High Performance Liquid Chromatography with UV Detection

High performance liquid chromatography with UV detection (HPLC–UV) consisted of two chromatographic pumps, Model 582 ESA (ESA Inc., Chelmsford, USA, working range 0.001–9.999 mL min^{-1}) and a reverse-

phased chromatographic column Zorbax eclipse AAA C18 (150 × 4.6; 3.5 μm particle size, Agilent Technologies, Santa Clara, CA, USA) and an UV detector (Model 528, ESA). Sample (20 μL) was injected automatically using (Model 542, ESA) which has the thermostated space for chromatographic column. Flow rate of mobile phase was set to 1 mL min^{-1}. The system was controlled by Clarity Software (Version 1.2.4, Prague, Czech Republic). The mobile phase consisted of A: methanol (HPLC grade purity, Sigma Aldrich) and B: distilled water (Mili Q, Millipore-Merck, Germany). The back pressure on the column was owing to applied conditions (temperature, composition of mobile phases between 110 and 160 bars). The other conditions that were optimized are shown in the "Results and Discussion". Chromatograms were recorded at wavelengths 270, 288, 530 and 630 nm and processed using Clarity software.

Ion Exchange Chromatography

For determination of Taurine, an ion-exchange liquid chromatography (Model AAA-400, Ingos, Prague, Czech Republic) with post-column derivatization with ninhydrin and VIS detector was used. A glass column with inner diameter of 3.7 and 350 mm in length was filled manually with a strong cation exchanger in sodium cycle LG ANB (Ingos) with approximately 12 μm particles and 8 % porosity. The column was tempered within the range from 40 to 115 °C. A double channel VIS detector with inner cell of volume 5 μL was set to two wavelengths, 440 and 570 nm. A solution of ninhydrin (Ingos) was prepared with 75 % (*v*/*v*) methylcelosolve (Ingos) and with 2 % (*v*/*v*) 4 M acetic buffer (pH 5.5). Tin chloride ($SnCl_2$) was used as a reducing agent. The prepared solution of ninhydrin was stored under an inert atmosphere (N_2) in dark at 4 °C. The flow rate of mobile phase was 0.25 mL min^{-1} and flow rate of ninhydrin was tested within the range from 0.1 to 0.35 mL min^{-1}. The reactor temperature was optimized within the range from 90 to 130 °C.

Recovery

Recovery of taurine was evaluated with homogenates [urine (male 25 years), commercially achieved energy drink and tea leaves] spiked with standard. Before extraction, 100 μL taurine standard and 100 μL water were added to samples. Homogenates were assayed blindly and taurine concentrations were derived from the calibration curves. Calculation of recovery was carried out as indicated by Causon [42] and Bugianesi et al. [43].

Estimation of Detection Limit

The detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner [44], whereas N was expressed as standard deviation of noise determined in the signal domain unless stated otherwise.

Results and Discussion

Spectrophotometric Characteristic of Taurine Complexes

Complex of Taurine with Sodium Hypochlorite and Phenol

The first of the studied complexes originated from reaction between taurine and sodium hypochlorite in the phosphate buffer (pH 10.35). This reaction is shown in Fig. 2a. Firstly, taurine reacts with sodium hypochlorite to give taurine-chloramine. Taurine-chloramine reacts with phenol and an intermediate 4-chloroiminocyclohexa-2,5-dien-1-one is probably formed (Fig. 2a). This intermediate product undergoes condensation reaction with another molecule of phenol and blue indophenol dye can be observed as a reaction product. Further, part of this blue complex undergoes additional condensation reaction or there occurs a reaction with sulfonic groups and a compound giving violet color to the solution that is formed. Spectrophotometric record of this reaction is demonstrated in Fig. 3a A1 a, b. Primarily, spectra of NaOCl and phenol were determined (Fig. 3a-A2). This spectrum shows only insignificant spectrophotometric changes, because phenol provides relatively narrow absorption maximum at about 270 nm and sodium hypochlorite wide absorption band with maximum at about 300 nm. Due to the reaction between taurine and halogenides, we observed significant changes in the absorbance of reactants phenol and sodium hypochlorite at $\lambda = 280$ nm (Fig. 3a a–g) and formation of above-described blue complex (indophenol dye) at the maximum of $\lambda = 630$ nm (indophenol dye, Fig. 3a A1 a, b). We found that higher concentrations of taurine (100 mM and higher) caused formation of new condensation product as complex of violet color at $\lambda = 530$ nm (condensation product, Fig. 3a A1 a, b), which has not been described yet. Changes in absorption maximum at $\lambda = 530$ nm were probably caused by arising condensation product, however, these questions will be published elsewhere.

Complex of Taurine with Sodium Hypobromite

The second of the studied complexes was formed by the reaction between taurine and sodium hypobromite (Fig. 3b). Primarily, we recorded spectrophotometric record of sodium hypobromite, where absorption maximum was detected at $\lambda = 332$ nm (Fig. 3b B2 a), and of taurine (Fig. 3b B2 b). Hypobromite was formed by a reaction of sodium bromide with sodium hypochlorite and hydrochloric acid and secondly, hypobromite reacts with

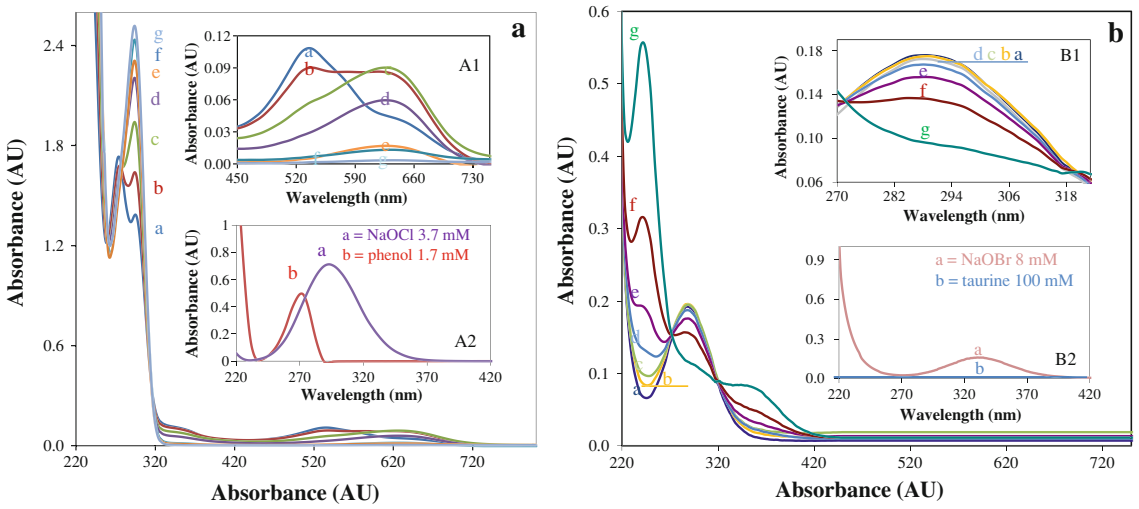

Fig. 3 **a** Spectra of taurine with phenol and sodium hypochlorite. Measured after 24 h of interaction at 22 °C. *A1* Complex formed at 530 nm (*a*, *b*) and 630 nm (*c*–*g*). *A2* Spectra of sodium hypochlorite (*a*) and phenol (*b*). **b** Spectra of taurine with sodium hypobromite. Measured after 30 min of interaction at 10 °C. *B1* Complex formed at 288 nm. *B2* Spectra of sodium hypobromite (*a*) and taurine (*b*)

taurine to give yellow-colored taurine-bromamine. Significant changes in absorbance at $\lambda = 232$ nm (Fig. 3b a–g) were observed. Reaction of taurine with sodium hypobromite led to the formation of complex with absorption maximum at $\lambda = 288$ nm (Fig. 3b B1 a–g).

Monitoring the Spectral Course under Different Temperature and Time of Interaction

Time and temperature dependences were other monitored parameters in our experiment. We investigated the effect of these parameters on the absorbance of studied complexes of taurine, respectively, effect of these parameters on courses of complexes formed during interactions between taurine, phenol and hypochlorite at $\lambda = 630$ nm and taurine and hypobromite at $\lambda = 288$ nm in the concentration range from 0.125 to 4 mM, temperature range from 10 to 60 °C and times of interaction from 0 to 30 min. Records were monitored at the 5 min long intervals. For both complexes studied, taurine and phenol with hypochlorite and taurine with sodium hypobromite, significant dependence on the concentration of taurine, reaction temperature and time of reaction was observed.

Complex of Taurine with Sodium Hypochlorite and Phenol

In addition, we investigated the effect of the time of the interaction on the change in the absorbance of complex of taurine with sodium hypochlorite and phenol. Values of absorbance increased with the increasing time of interaction. The most evident increase in absorbance was detected within the time interval 0–5 min, after it, increase in absorbance was only moderate. The most significant changes were observed under the highest applied concentration 4 mM (increase for 1.52 AU at 60 °C). In the next step, we monitor the effect of temperature on the changes in absorbance, it means on the changes in color of investigated complex. The increasing temperature during interaction led to the increase in absorbance of studied complex. The most significant differences were (similar to the time dependences) observed at the highest applied concentration (4 mM). For this complex, the increase in temperature from 10 to 60 °C in the time interval from 0 to 30 min caused the increase in the absorbance for more than 200 % at all tested concentrations of taurine. The analytical parameters are shown in Table 1.

Table 1 Table summarizing analytical parameters of determination of the complexes using spectrophotometry $n = 3$

	Spectrophotometry					
	Limit of detection (μM)	Determination coefficient	Reaction time (min)	Optimum temperature (°C)	Measuring range (μM-mM)	Absorption maximum (nm)
Taurine + phenol and hypochlorite	10	$r^2 = 0.992$	30	60	10–100	530 and 630
Taurine + hypobromite	10	$r^2 = 0.970$	1	10	10–100	288

Chromatographic Analysis of Taurine Complexes

The separation of the complexes might bring the new information about these complexes; however, HPLC has never been used for analysis of taurine complexes. Using HPLC for studying of taurine complexes, we can obtain the approach, which might be useful for subsequent mass spectrometric method or similar other types of complex characterization. Therefore, taurine complexes were analysed using HPLC–UV at 22 °C with the focusing on the separation of all studied complexes, which were found in the previous spectrophotometric measurements.

Basic Characterization

Primarily, we performed basic characterization of individual components and complexes. Taurine itself demonstrated no absorbance within the studied range from 220 to 800 nm. Sample (40 µL) was injected using autosampler at 22 °C. Mobile phase glow rate was 1 mL min^{-1}. Mobile phase was consisted from A: methanol and B: distilled water in ratio 60/40, *v*/*v* (Fig. 4a). Sodium hypobromite was detected at $\lambda = 288$ nm in the retention time RT: 4.01 (40 µL sample, 22 °C, mobile phase flow rate 1 mL min^{-1}, mobile phase 50 %A/50 %B, *v*/*v*, Fig. 4b). Complex of taurine and sodium hypobromite (taurine-bromamine) was detected at $\lambda = 288$ nm in the retention time RT: 1.6 (40 µL sample, 22 °C, mobile phase flow rate 0.8 mL min^{-1}, mobile phase 10 %A/90 %B, *v*/*v*, Fig. 4c). Sodium hypochlorite demonstrated no detectable signal within the range from 220 to 800 nm under same conditions as in the case of sodium hypobromite (Fig. 4d). Absorbance of phenol was detected at $\lambda = 270$ nm in the retention time RT: 2.21 (40 µL sample, 22 °C, mobile phase flow rate 1 mL min^{-1}, mobile phase 60 %A/40 %B, *v*/*v*, Fig. 4e). Chromatograms measured at $\lambda = 630$ nm (Fig. 4f) show two distinct signals, which correspond to two different complexes of taurine with chlorinated phenol (indophenole—RT: 3.42; condensation product—RT: 4.03). The chromatogram was analysed under the following conditions (40 µL sample, 22 °C, mobile phase flow rate 0.8 mL min^{-1}, mobile phase 50 %A/50 %B, *v*/*v*). It clearly follows from the results obtained that separation of taurine complexes by the use of reversed-phase chromatography is possible at the chosen wavelengths. In addition, this method is selective as suitable for the purposes of further optimization.

Optimization of Separation of Taurine-Phenol-Hypochlorite Complex

The optimization of the separation of complexes of taurine–phenol–hypochlorite was carried out. We investigated the effect of the mobile phase composition and temperature during separation. Optimization of mobile phase composition

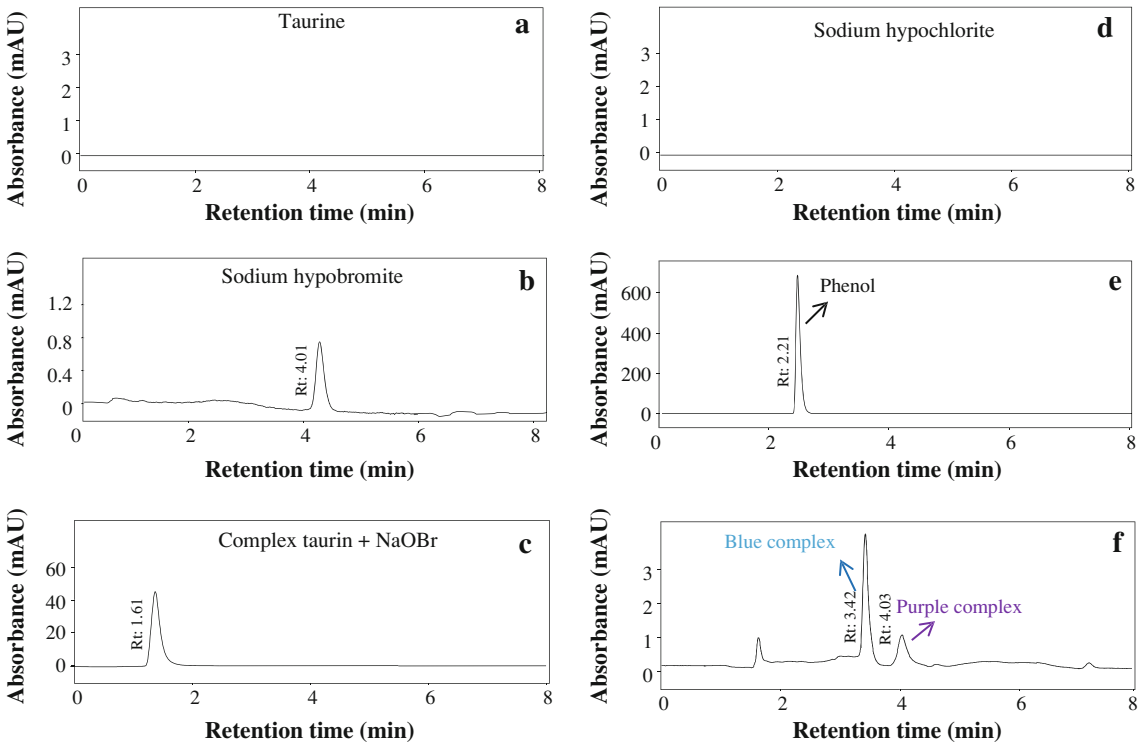

Fig. 4 Typical HPLC UV chromatograms of **a** taurine, **b** sodium hypobromite, **c** complex taurine + NaOBr, **d** sodium hypochlorite, **e** phenol and **f** indophenole and its condensation product

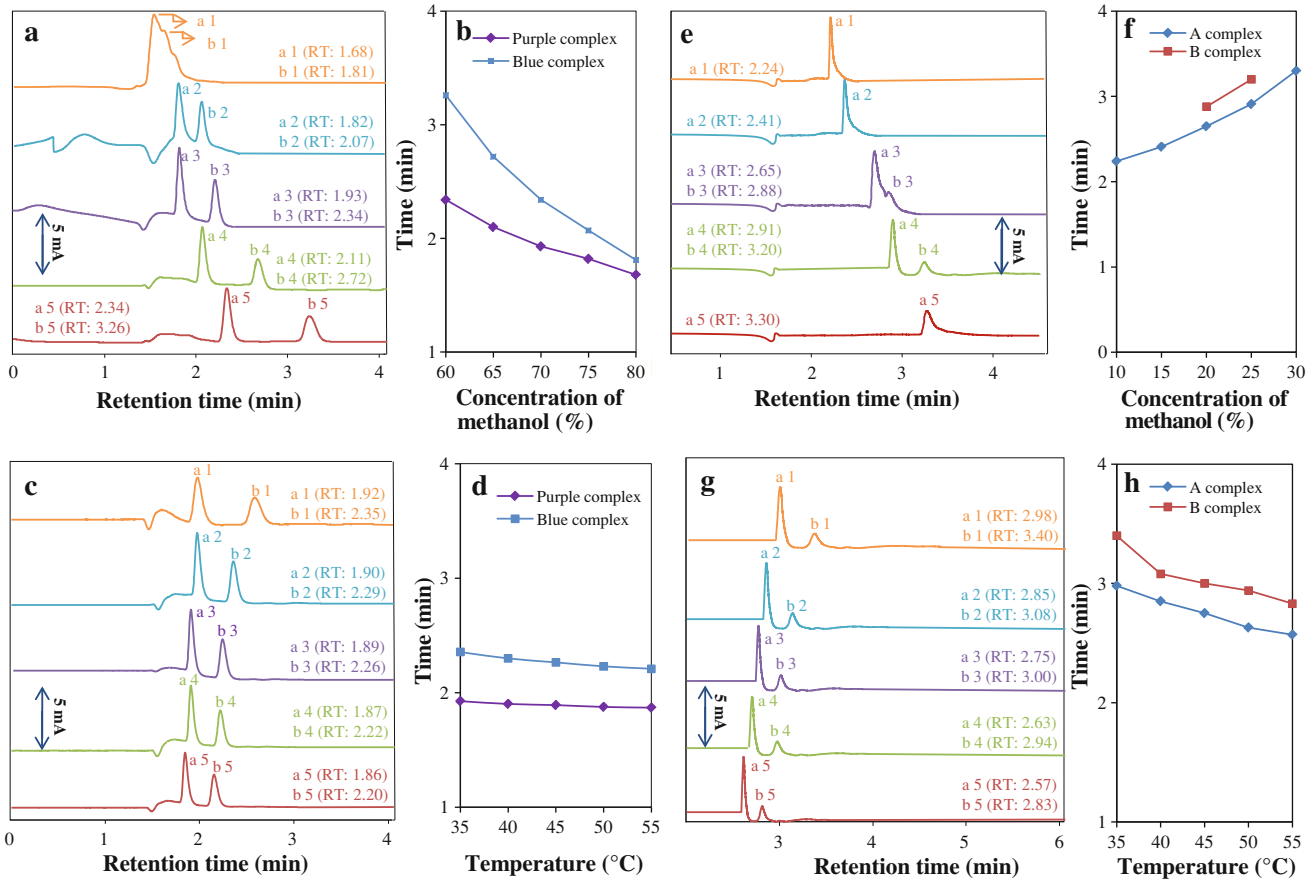

Fig. 5 The influence of methanol content in the mobile phase (60, 65, 70, 75 and 80 %, *v*/*v*) on taurine–phenol–hypochlorite complexes (*blue* and *violet*), **a** typical chromatograms and **b** the influence on retention times of the complexes. The influence of temperature (35, 40, 45, 50 and 55 °C) on taurine–phenol–hypochlorite complexes, **c** typical chromatograms and **d** the influence on retention times of the complexes. The influence of methanol content in the mobile phase (10, 15, 20, 25 and 30 %, *v*/*v*) on taurine-bromamine complexes (*a* and *b*), **e** typical chromatograms and **f** the influence on retention times of the complexes. The influence of temperature (35, 40, 45, 50 and 55 °C) on taurine-bromamine complexes, **g** typical chromatograms and **h** the influence on retention times of the complexes

was based on the gradual decreasing of methanol rate, always for 5 % within the range from 80 to 60 % in the mobile phase B. The most effective separation was achieved at the concentration 70 % B, where retention time of signals (a) and (b) were 1.93 and 2.34 min, respectively (Fig. 5a a3, b3). At the higher concentrations of methanol, co-elution occurred. On the other hand, lower concentrations of methanol led to the prolongation of the time of analysis. In addition, signal broadening was evident. The influence of peak heights of the complexes on methanol concentration is shown in Fig. 5b.

Optimization of temperature parameters was carried out under the most suitable parameters determined in previous optimization, i.e. at the methanol concentration of 70 % (Fig. 5c). Effect of temperature on separation was investigated within the range from 35 to 55 °C for 5 °C (Fig. 5d). It is well evident from the obtained results that retention time of signal (a) is not changed with temperature. On the other hand, signal (b) is significantly changed with temperature. The most rapid and effective separation was determined at 55 °C (Fig. 5c a5, b5) Taurine–phenol–hypochlorite complex (200 μM) was effectively separated using reversed-phase chromatography, where two distinct, well-separated signals corresponding to violet and blue complexes characterized above were obtained at the most suitable chromatographic conditions (isocratic elution, mobile phase water:methanol 30:70 %, *v*:*v* and 55 °C). The parameters of the method are shown in Table 2. After optimization of the method, the calibration curve of taurine within the range from 1.25 to 100 μM was measured. The good linearity was estimated with $r^2 = 0.99$. The limit of detection (3 S/N) was estimated as 1 μM. Other analytical parameters are shown in Table 3.

Optimization of Separation Taurine-Bromamine Complex

Separation of taurine-bromamine complexes was carried out with regard to expected less hydrophobicity of the whole complex, thus the separation conditions had to be reconsidered. Similar to separation of taurine-phenol-hypochlorite complexes, the effect of the composition of mobile phase and

temperature was investigated (Fig. 5e). Optimization of composition of mobile phase was based on the gradual decrease of methanol rate for 5 % within the range from 30 to 10 % of mobile phase B (Fig. 5f). It was possible to insufficiently separate individual signals at 20 % of methanol and sufficiently at 15 % of methanol. The best separation of signals was determined at the 15 % concentration of methanol, where the retention time of signal (a) was 2.91 min and of signal (b) 3.2 min (Fig. 5e a4, b4). Optimization of temperature was carried out under this condition, i.e. methanol concentration 15 % (Fig. 5g). Temperature was increased identically as it is described in the previous subchapter. It clearly follows from the results obtained that retention time of signals linearly decreased with the increasing temperature. The most rapid separation was achieved at 55 °C, where both signals were well separated (Fig. 5g a5, b5). However, this phenomenon was observed in all three highest temperatures. It was possible to separate taurine-bromamine complex (200 μM) using the reversed-phase chromatography, where two well-separated signals correspond to signals described in previous chapters were observable under the most suitable chromatographic conditions (isocratic elution, mobile phase water:methanol 85:15 % *v*:*v* and 55 °C). The parameters of the method are shown in Table 2. For estimating the method the calibration curve of taurine in range 1.25–100 μM was analysed. The calibration curve shows good linearity with the $r^2 = 0.99$. The limit of detection (3 S/N) was estimated as 1 μM. Other analytical parameters are shown in Table 3.

Real Samples and Recovery of Determination

We were also interested in the recovery of the optimized procedure. The recovery was analysed under the optimized conditions. The one sample of urine (male 25 years), commercially achieved energy drink and tea leaves eluate were determined. The samples, 25 μM taurine and spiked samples with 25 μM taurine were analysed. The obtained results indicated the good recovery of the method varied between 79 and 86 % ($n = 3$, Table 4). These results suggest the purpose of this sensitive method to analyse the taurine in biological samples.

Optimization of Taurine Determination using Ion Chromatography

Further, we aimed our attention at investigating the ability of the above-characterized taurine and taurine complexes to scavenge reactive oxygen species. For this purposes, ion chromatography with post-column derivatization using ninhydrin (2,2-dihydroxy-1,3-indandion) and UV detection at 570 nm was used [36, 37]. The effect of the increasing reactor temperature (90–130 °C, Fig. 6a, b) and column temperature (40–80 °C, Fig. 6c, d) on taurine was investigated per 5 °C steps. There was also tested the influence of post-column flow rate of ninhydrin (0.1–0.35 mL min^{-1}, 0.05 mL min^{-1}, Fig. 6e, f) on taurine complexes. The temperature in the reaction space plays a key role in the formation of violet complex [45]. Our results indicate that the reaction equilibrium shifts to the product with increasing temperature (Fig. 6a). The heights of the detected peaks increased with the increasing temperature gradually up to 100 °C and then the great increase occurred. The slight decrease of the signals was determined in the range of temperature from 120 to 130 °C. It clearly follows from the results obtained that 110 °C was the optimal temperature for further experiments (Fig. 6a, b). Temperature of the chromatographic column was another

Table 2 Table summarizing method parameters of determination of the complexes using HPLC–UV

	HPLC–UV					
	Limit of detection (μM)	Reaction time (min)	Optimum temperature (°C)	Retention time (min)	Measuring range (μM-mM)	Absorption maximum (nm)
Taurine + phenol and hypochlorite	10	30	55	2	10–50	530
Taurine + hypobromite	10	1	55	3	10–50	288

Table 3 The analytical parameters of chromatographic determination of taurine-phenol-hypochlorite and taurine-bromamine complexes

Complex	Regression equation	Linear dynamic range (μM)	r^2	LOD (μM)	LOD[a] (fmol)	LOQ (μM)	LOQ[a] (nmol)	RSD (%)
Taurine–phenol–hypochlorite	$y = 0.0718x - 1.0205$	1.25–100	0.999	1	0.2	4	2	2.50
Taurine-bromamine	$y = 0.1095x - 0.7795$	1.25–100	0.995	1	0.2	4	4	3.70

[a] Per injection

Table 4 The recovery (%) of both methods optimized for taurine detection in real samples

Sample	Taurine–phenol–hypochlorite	Taurine-bromamine
Urine	75	69
Energy drink	89	78
Tea leaves eluate	92	86

tested parameter. The temperature can significantly affect the retention of some analytes, but, in our case, there was no determination of any change in RT of taurine within the tested temperature range from 40 to 80 °C (Fig. 6c, d). Due to the greater tendency of ionic stationary phase for shrinkaging at higher temperatures (app. 80 °C), we used 60 °C for further experiments. Flow rate of ninhydrin was the last tested parameter. The increasing flow rate caused the increase in taurine peak (Fig. 6e, f), which was very fast from 0.1 to 0.3 mL min^{-1}. At flow of 0.35 mL min^{-1}, the signal began to decrease (Fig. 6f). The initial almost linear increase of the signal related to the saturation of the output liquid chromatographic column with ninhydrin. The optimal flow rate of this dye was 0.25 mL min^{-1}.

Simulation of Oxidative Stress by Peroxide

There were carried out three series of simulations (eight samples) of oxidative stress by peroxide (1.72, 3.34, 6.85, 13.75, 27.5, 55, 110 and 220 mM). Individual concentrations of peroxide were diluted with taurine and its complexes concentrations of 0.008, 0.02, 0.03, 0.063, 0.125, 0.25, 0.5 and 1 mM. The dependences of peak heights of taurine and its complexes on the concentration of the compound of interest are shown in Fig. 7a–c. We demonstrated a significant antioxidant effect of taurine and its complexes with phenol, hypochlorite and/or hypobromite (Fig. 7d). Taurine itself reacts with peroxide more intensely than in a bound form, which can be associated with the highest signal decrease. Complexes stabilized structure taurine against peroxide radicals, resulting in slower decreasing of peak heights. The most stable was taurine complexes with phenol and hypobromite. These results confirm that taurine complexes with phenol and hypobromite have significant antioxidant effects. In addition, TauBr and TauCl are major haloamines generated by eosinophils and neutrophils at a site of inflammation. Both haloamines share anti-inflammatory and anti-oxidant properties. TauBr, similar to TauCl, decreases the production of proinflammatory mediators. Their anti-inflammatory and anti-oxidant activities are enhanced by their ability to induce the expression of heme oxygenase-1

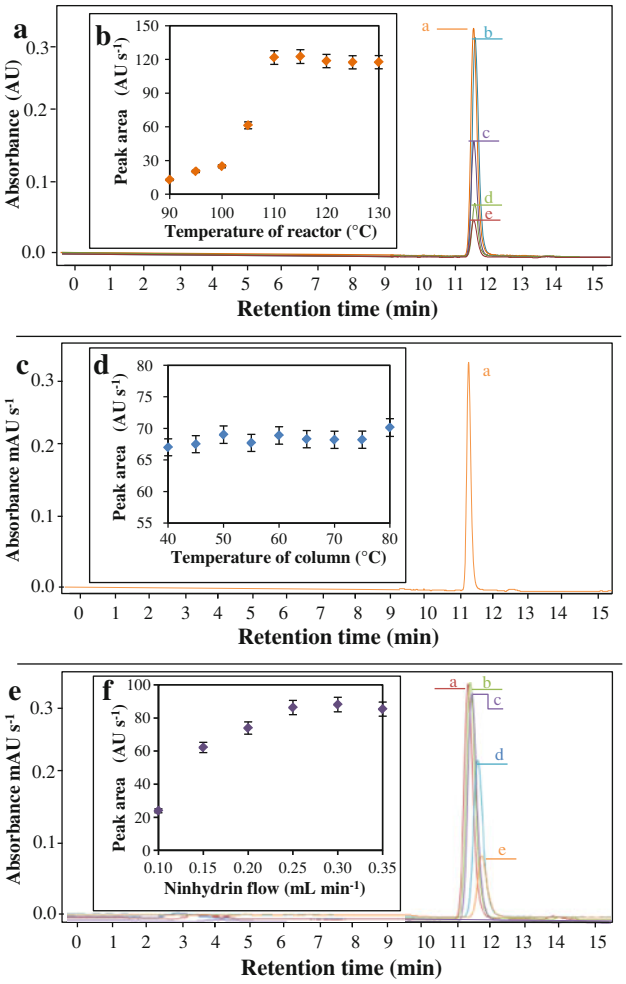

Fig. 6 **a** Typical HPL chromatograms of taurine (1 mM) measured by ionex chromatography with post-column derivatization with ninhydrin at various temperature in reactor (90–130 °C). **b** The effect of tested temperature interval on taurine peak area. **c** Typical chromatograms of taurine (1 mM) measured by ionex chromatography with post-column derivatization with ninhydrin at various temperature on column (40–80 °C). **d** The effect of tested temperature interval on taurine peak area. **e** Typical chromatograms of taurine (1 mM) measured by ionex chromatography with post-column derivatization with ninhydrin at various flow rates of ninhydrin (0.1–0.35 mL min^{-1}). **f** The effect of tested flow rates interval on taurine peak area

(HO-1) [1]. Taurine chloramine is the major chloramine generated in activated neutrophils via the reaction between the overproduced hypochlorous acid and the stored taurine. Taurine chloramine has anti-inflammatory and cytoprotective effects in inflamed tissues by inhibiting the production of inflammatory mediators. Taurine chloramine increases heme oxygenase activity and also protects against hydrogen peroxide (H_2O_2)-derived necrosis in macrophages [46, 47].

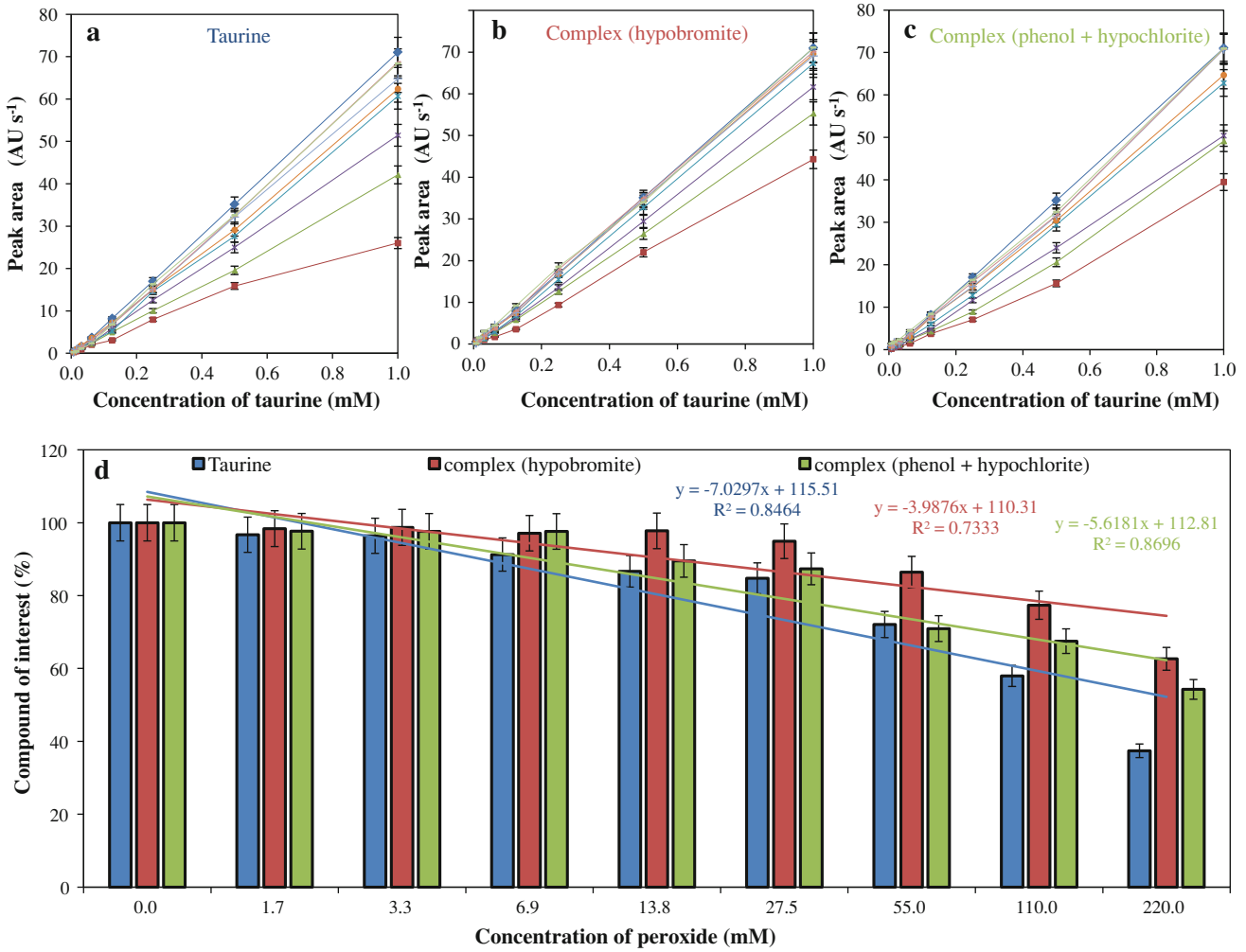

Fig. 7 **a** Taurine (0.008, 0.02, 0.03, 0.063, 0.125, 0.25, 0.5 and 1 mM) was mixed with peroxide (1.72, 3.34, 6.85, 13.75, 27.5, 55, 110 and 220 mM) and the peak of taurine was determined. **b** Complex of taurine with hypobromite (0.008, 0.02, 0.03, 0.063, 0.125, 0.25, 0.5 and 1 mM) was mixed with peroxide (1.72, 3.34, 6.85, 13.75, 27.5, 55, 110 and 220 mM) and the peak of the complex was determined. **c** Complex of taurine with phenol and hypochlorite (0.008, 0.02, 0.03, 0.063, 0.125, 0.25, 0.5 and 1 mM) was mixed with peroxide (1.72, 3.34, 6.85, 13.75, 27.5, 55, 110 and 220 mM) and the peak of the complex was determined. **d** Comparison of changes in the heights of peaks of taurine and its complexes affected by peroxide

Conclusion

Our work demonstrates that taurine-bromamine and taurine–phenol–hypochlorite complexes are suitable complexes for the determination of taurine using the HPLC–UV and spectrophotometric methods. The benefit of this study is based on the establishment of the fact that taurine is able to form different complexes (blue $\lambda = 630$ nm and violet $\lambda = 530$ nm) with phenol and hypochlorite. Further, we evaluated the ability of taurine complexes to scavenge reactive oxygen species.

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