Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1283–1289
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of selenium(IV) in pharmaceuticals and premixes by micellar liquid chromatography

Artem U. Kulikov

Laboratory of Pharmacopoeial Analysis, Scientific and Expert Pharmacopoeial Centre, Astronomicheskaya street 33, 61085 Kharkov, Ukraine

Received 8 August 2006; received in revised form 4 October 2006; accepted 20 October 2006
Available online 5 December 2006

Abstract

A simple micellar liquid chromatographic technique for the determination of selenium(IV) in pharmaceutical products (multi-vitamin tablets, syrups) and animal premixes after pre-column derivatization with 2,3-diaminonaphthalene was developed and validated. Hypersil ODS column, 10% (v/v) 1-butanol in 0.05 M sodium dodecyl sulfate as the mobile phase and UV detection at 378 nm and were used. The retention time was about 8 min. In the course of the validation study, the specificity of the method was demonstrated. Linearity was established in the range 0.33–3.3 μg ml^{-1} of selenium(IV) content. The limits of detection and quantitation were 0.1 and 0.3 μg ml^{-1}, respectively. The method showed excellent accuracy (100.04%). Precision (repeatability) gave a relative standard deviation less than 1%. The influence of the various method parameters (robustness study) was also investigated.

Keywords: Micellar liquid chromatography; Selenium(IV); 2,3-Diaminonaphthalene; Naphthylpiazselenol; Sodium dodecyl sulfate

1. Introduction

Micellar liquid chromatography (MLC) is a type of chromatography that is an alternative for reversed-phase liquid chromatography (RP-HPLC) [1–6]. MLC is successfully applied to analysis of drugs, biological fluids [1–3] and in environmental analysis [7]. In addition to the number of compounds with a wide range of polarities amenable to separations by RP-HPLC, MLC further extends the range of analytes to almost all hydrophobic and many hydrophilic compounds. Other advantages of MLC over RP-HPLC are the possibility of simultaneous separation of both ionic and non-ionic compounds without the need for gradient elution, low cost, low toxicity and low volatility of the mobile phase and improved detection sensitivity. Also MLC shows better selectivity than HPLC [1], based on its unique three-way equilibrium mechanism [8] where micelles act as a pseudo phase in addition to the mobile and stationary phases.

In recent years, hybrid micellar eluents are widely used in MLC, which are aqueous solutions of surfactants with a concentration above critical micelle concentration (CMC) that contains the organic solvent additives, such as aliphatic alcohols, acetonitrile and tetrahydrofuran. It has been found, that organic additives increase the eluting strength and modify the selectivity [1,7].

Selenium is an essential trace element (constituent of glutathione peroxidase) for plants, animals and human. Its physiological nature in human is ambivalent since it can cause disease by its deficiency, but it is toxic at levels relatively close to those required for health [9].

The main sources of selenium for human and animals are plants, which get selenium from the soil. But now in many regions human and animals obtain it in small amounts with foodstuff and the deficiency of selenium in organism is filled up by administration of multi-vitamin drugs with mineral complexes or special nutritional dietary supplements. Usually selenium as microelement in multi-vitamin drugs comes in the form of sodium selenite or selenous acid, and its content is about 1–10 μg ml^{-1} in syrup or 10–50 μg per tablet. Premixes, as animal fodder, contain about 10–100 mg selenium per killogram.

The most commonly used methods for selenium determination are ICP-MS methods [10,11], AAS with using hydride generation system or graphite cell [12–14], atomic fluorescence spectroscopy [15] and spectrofluorometric methods after derivatization [16,17]. Sun et al. [18] proposed capillary electrophoresis methods and Stoica et al. [19] used differential pulse

E-mail address: kulikov@phukr.kharkov.ua.

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.10.041

cathodic stripping voltammetry for selenium determination in pharmaceutical products.

The use of gas and liquid chromatographic methods for selenium determination were also developed and reviewed by Uden [20] and Sarzanini and Mentasti [14].

Milano et al. [21] proposed short-time HPLC–UV irradiation–hydride generation–quartz cell AAS methods for the determination of organic and inorganic selenium compounds. Do et al. [22], Jen et al. [23] and Gergely et al. [24] used ion-pair RP-HPLC for the determination of selenium compounds (selenite, selenate, seleno amino acids) in the environment (lake water), in mammals, mushroom proteins, etc.

Katavarupu Yathavakilla et al. [25] and Goessler et al. [26] used strong cation-exchange chromatography with ICP-MS and Liang et al. [27] used anion-exchange chromatography with AFS for determination of selenium compounds. Chatterjee et al. [28,29] developed a HPLC method coupled with high-power nitrogen microwave-induced plasma (MIP) mass-spectrometry and gel-permeation chromatography with ICP-MS for the determination of selenite, selenate and selenoorganic compounds in urine samples.

Direct chromatographic methods for inorganic selenium determination without the use of special detectors are problematic, because of their low sensitivity. Therefore, derivatization reagents such as 1,3-dibromo-5,5-dimethylhydantoin [30], dithizone [31], benzylpropionitrile dithiocarbamate [32], 1,2-diaminobenzene [33] were used.

In the present work, we developed and validated a micellar liquid chromatography method with UV detection for quantitative analysis of selenium(IV) in pharmaceutical preparations (multi-vitamin syrups, tablets, capsules) and premixes after pre-column derivatization with 2,3-diaminonaphthalene. The method is rapid, facile and environmentally benign because of using low amounts of toxic organic solvents.

2. Experimental

2.1. Reagents

Sodium selenite (99% quality, Aldrich, Milwaukee, WI, USA), 2,3-diaminotaphtalene (98.0% quality, Sigma–Aldrich Chemie, Germany), 1-butanol (1-BuOH), 2-butanol (2-BuOH), *iso*-butanol (2-methylpropanol-1, *iso*-BuOH), *tert*-butanol (*tert*-BuOH), 1-pentanol and sodium dodecyl sulfate (SDS), all were purchased from Fluka Chemie (Buchs, Switzerland). Hydrochloric acid and *n*-heptane were from Merck (Darmstadt, Germany). Double distilled water was used in all the experiments.

2.2. Apparatus

The chromatographic measurements were carried out with Hewlett Packard equipment (Agilent Technologies, Waldbronn, Germany) consisting of a Series 1050 pump, a Series 1050 variable wavelength UV detector and a Series 3395 integrator. The analytical column was Hypersil ODS (150 mm × 4.6 mm, 5 μm; Merck KgaA, Darmstadt, Germany). The spectrophotometric studies were carried out using HP 8453 UV–vis spectrophotometer (Agilent Technologies, Waldbronn, Germany). The pH value was determined with a Beckman Φ-200 pH meter (Beckman Instruments, Fullerton, CA, USA).

2.3. Chemicals

Preparation of 2,3-diaminonaphtalene reagent: about 0.1 g of 2,3-diaminonaphthalene was placed into 100 ml volumetric flask, added about 50 ml of 1 M hydrochloric acid and stirred for about 30 min. The solution was diluted to volume with the same solvent, placed into 250 ml separating funnel, added about 20 ml of *n*-heptane, shaken vigorously for 10 min and was left till the separation of the layers. The upper layer was then removed, and the aqueous solution of the reagent was transferred into a dark bottle and used for pre-column derivatization. The reagent was used within 14 days if stored at about 5 °C in the dark.

2.4. Standard preparation

Stock standard solution of the selenium was prepared by weighting 0.1450 g of sodium selenite in a 200 ml volumetric flask, dissolving in water and diluting to volume with the same solvent. A 1.0 ml of this solution contains 331.1 μg of selenium. The solution was stored at approximately 5 °C in the dark for 5 days.

Working standard solutions were prepared by dilution of the selenium stock standard solution with 0.1 M hydrochloric acid solution to obtain five different concentrations within the range of interest (0.331, 0.993, 1.66, 2.32 and 3.31 $\mu g\,ml^{-1}$). These solutions were used for linearity investigation.

Reference standard solutions were used for evaluation of precision, repeatability and accuracy. For this purpose aliquots of selenium stock standard solution were diluted to obtain five different concentrations: 0.795, 0.894, 0.993, 1.093 and 1.192 $\mu g\,ml^{-1}$. These solutions contained 80, 90, 100, 110 and 120% of nominal amount of selenium (1 $\mu g\,ml^{-1}$ Se(IV) as Na_2SeO_3) in syrup. Solutions were freshly prepared before use.

2.5. Derivatization procedure

The derivatization procedure for the selenium determination was based on the method described in literature [16,17]. Twenty tablets were weighted and powdered. Premix powder was thoroughly triturated.

For samples: an amount of the powdered tablets, capsules, premixes and syrup, equivalent to 10 μg of Se(IV) was accurately weighted, placed in 50 ml volumetric flask, added about 30 ml of 1 M hydrochloric acid, sonicated for approximately 5 min, added 5.0 ml of 2,3-diaminonaphthalene reagent, stirred for some minutes, kept on the boiling water bath for 10 min and cooled to room temperature. A 3.0 ml of *n*-heptane was added, the solution was shaken vigorously for 2 min and was left till to the separation of the layers. The upper layer was filtered with 0.45 μm filter for HPLC analysis and used for selenium determination.

For standards: 10.0 ml of selenium working standard solutions or reference standard solutions were placed into 50 ml volumetric flask, added about 30 ml of 1 M hydrochloric acid, followed as described above for sample preparation.

2.6. Chromatographic separation

A 0.05 M solutions of SDS with 10% (v/v) of organic modifiers (butanols) were prepared and used as mobile phases at $40.0 \pm 0.1\,^{\circ}C$. The flow rate was $1.0\,ml\,min^{-1}$ and the injected volume was 25 μl. The detection wavelength was 378 nm.

2.7. Data sources, software and processing

The statistical analyses were performed with Microsoft Excel (2002, Microsoft Corporation, http://office.microsoft.com).

3. Results and discussion

3.1. Development of the derivatization technique

The first aim of this work was to increase the sensitivity and selectivity of the determination of selenium in pharmaceutical products by pre-column derivatization using 2,3-diaminonaphthalene as the chromogenic reagent. In Fig. 1 the reaction of selenite ion or selenous acid with 2,3-diaminonaphthalene in acid medium is shown.

The product of this reaction is the highly fluorescent 4,5-naphthylpiazselenol. According to the literature [16], Se(IV) quantitatively reacts with 2,3-diaminonaphthalene at pH about 1. Organic solvents such as chloroform, ethers, hexane, cyclohexane, etc. can be used for the extraction of the reaction product.

Absorption spectra of 4,5-naphthylpiazselenol were measured both in organic solvent (*n*-heptane) and in micellar mobile phase. The absorbance maxima were at 222, 261 and 378 nm in all cases. Three hundred and seventy-eight nanometer was selected as the analytical wavelength.

Selenium quantitatively reacts with 2,3-diaminonaphthalene at room temperature during 2–3 h [16]. As described in literature [17], heating can accelerate the reaction, but this was not recommended because in this case the rate of the air oxidation of 2,3-diaminonaphthalene also increases and this influences the spectrophotometric analysis. In MLC technique we could separate peaks of the oxidation products of 2,3-diaminonaphthalene from 4,5-naphthylpiazselenol peak. Hence, we optimized the derivatization procedure and proposed heating of the reaction mixture for 10 min on boiling water bath for reaction completeness. Then we extracted the reaction product with 3 ml of *n*-heptane (the extraction completeness was controlled and was about 99%). This solution was injected into the chromatograph

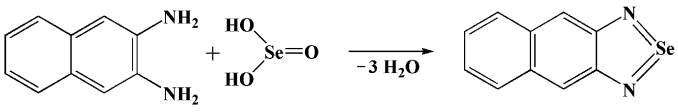

Fig. 1. Selenium derivatization with 2,3-diaminonaphthalene.

that allows eliminating some steps – drying to residue and dissolving in the mobile phase – that was necessary if we worked with RP-HPLC.

3.2. Development of the micellar liquid chromatography methods

The optimum mobile phase was obtained through the application of an interpretive procedure, which needs the solute retention data and considers the parameters of the chromatographic peak in the selected mobile phases of surfactant, adequately distributed in the linear variable space. It should be taken into account that at high concentration of the surfactant the chromatographic efficiency deteriorates. This interpretive procedure was applied to the optimization of the composition of hybrid micellar solutions of surfactant and organic modifier.

The retention time of 4,5-naphthylpiazselenol was to high with purely micellar mobile phases. It was more than 60 min when 0.05 or 0.10 M of SDS solutions were used. The high hydrophobicity of this compound ($\log P_{O/W}$ is about 3.5 calculated using ACD/Labs Software V. 4.01/11, Advanced Chemistry Development, Inc., Toronto ON, Canada) made it to be strongly associated with the modified alkyl chains of the stationary phase. The use of hybrid micellar mobile phase (with an added modifier) was thus necessary to reduce the retention. Butanol (normal and iso) and pentanol were selected as the most adequate modifiers, since they give higher elution strength than the short chain alcohols [1]. The effect of the reduction of retention produced by butanol or pentanol was larger at increasing amounts of the alcohol in the mobile phase. The presence of alcohol in micellar mobile phase leads to the formation of associate solute-micelle in the bulk aqueous phase. This results in reduction in the capacity factor.

The influence of type and amount of organic modifier on two basic parameters including the number of theoretical plates (*N*) and asymmetry factor (*B*/*A*) was investigated in the range of 1-butanol (1–20%) step 5% and 1-pentanol (1–5%) step 2%. The results are presented in Table 1. The results show that a considerable improvement in chromatographic efficiency was obtained by addition of more than 10% of 1-butanol. Mobile phase with 1-pentanol shows high asymmetry factor and lower efficiency.

It has been reported [34] that changing the type of organic modifier, for example use of normal or iso alcohols, can affect the chromatographic behavior of solutes when micellar mobile phases are used. We investigated the influence of *iso*-butanols as organic modifiers on 4,5-naphthylpiazselenol chromatographic behavior and its asymmetry factor and on the column efficiency (Table 1). As is apparent from Table 1, mobile phases containing 2-butanol and *iso*-butanol showed practically the same column efficiency and asymmetry factor, and can be used as alternative organic modifier.

The efficiency of the column could be improved at high temperatures due to faster mass transfer of solute between mobile and stationary phases. The most appropriate temperature is 40 °C for determination of 4,5-naphthylpiazselenol.

Table 1
The effect of organic modifiers on the chromatographic performance of 4,5-naphthylpiazselenol

	k	$N \times 10^{3}$[c]	B/A
Organic modifier volume fraction[a]			
1% 1-butanol	16.55	3.1	2.54
5% 1-butanol	7.89	6.3	1.77
10% 1-butanol	4.53	12.4	1.38
15% 1-butanol	2.03	13.2	1.29
20% 1-butanol	1.02	17.6	1.15
1% 1-pentanol	9.05	3.8	2.24
3% 1-pentanol	6.82	6.7	1.53
5% 1-pentanol	3.11	9.4	1.41
Type of organic modifier[b]			
1-butanol	4.53	12.4	1.38
2-butanol	4.50	12.6	1.44
iso-butanol	4.66	10.9	1.60
tert-butanol	5.63	8.4	1.78

[a] SDS concentration is 0.05 mol l^{-1}.
[b] 0.05 M SDS; 10% (v/v) of organic modifier.
[c] Theoretical plates m^{-1}.

On the basis of the selected organic modifier and SDS concentration, an optimization study was carried out. The optimal conditions with acceptable efficiency and asymmetry factor were found to be 0.05 M SDS and 10% (v/v) of 1-butanol. Mobile phases with 0.05 M SDS and 15 and 20% (v/v) of 1-butanol show better efficiency (see Table 1), but in this case the 4,5-naphthylpiazselenol peak has small retention time (k is 2.03 and 1.02, respectively) and this is not acceptable for the quantitative analysis.

3.3. Validation of the MLC method

The procedures and parameters used for the validation of the chromatographic method are those described in the USP 29, chapter <1225> [35a], International Conference of Harmonization (ICH) Guidelines [36,37] and in the literature [38,39].

3.3.1. Specificity (selectivity)

The selectivity of the MLC method was assessed by the analysis of 4,5-naphthylpiazselenol oxidized with 10% H_2O_2 or stressed at short-wavelength UV light in an oven for 3 h as described in literature [37]. Fig. 2 shows that the peak of 4,5-naphthylpiazselenol was satisfactorily separated from the peaks of degradation or oxidation products. The peak purity was in all cases above 98%.

3.3.2. Linearity

Linearity was determined by analysis of a series of standards at five different concentrations that span 80–120% of the expected working range of the assay [36,37]. Adamovich [40] recommended a range spanning 25–200% of the nominal range of analyte, using standards and spiked placebo samples. A linear regression equation obtained from the results should have an intercept not significantly different from zero; if it is the case, it should be demonstrated that this has no effect on the accuracy of the method.

In this investigation, a linear plot was obtained from five different concentrations of selenium working standard solutions in the range 0.331–3.31 μg ml^{-1} using five replicate injections. The regression line was calculated as $Y = A + BX$, where X was

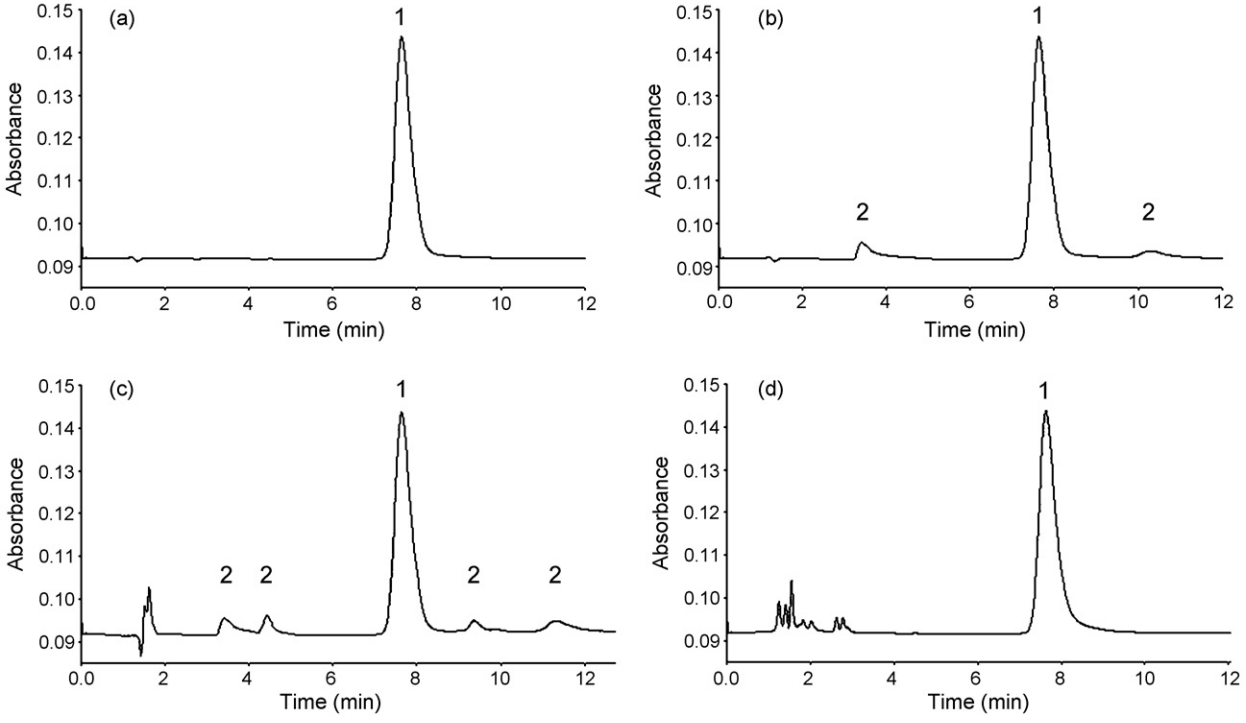

Fig. 2. Chromatograms obtained during assessment of specificity. (1) 4,5-Naphthylpiazselenol; (2) unknown impurities. (a) 4,5-Naphthylpiazselenol standard. (b) Sample of 4,5-naphtylnaphthylpiazselenol had been exposed to short-wavelength UV light for 3 h. (c) Sample of 4,5-piazselenol oxidized with 10% H_2O_2. (d) Chromatogram obtained for selenium determination in Ferramin-vita syrup.

Table 2
Results for determination of accuracy by analysis of the samples of known concentration

Se concentration in reference standard solutions (μg ml^{-1})	Experimental concentration was founded[a] (μg ml^{-1})	Recovery (%)
0.795	0.798	100.42
0.894	0.896	100.23
0.993	0.992	99.94
1.093	1.091	99.83
1.192	1.189	99.77

Mean value: 100.04; RDS (%): 0.34.
[a] Mean value of five determinations.

Table 4
Recovery data of Se(IV) added to a Ferramin-vita syrup

Se(IV) added (μg ml^{-1})	Se(IV) found[a] (μg ml^{-1})		Recovery (%)
	With addition	Without addition	
–	–	1.08	–
0.33	1.38	1.05	97.2
0.66	1.79	1.14	104.6
0.99	2.10	1.12	102.8
1.32	2.39	1.07	99.1

A sample of 10.0 ml was used.
[a] Mean of three determinations.

the selenium concentration (μg ml^{-1}) and Y was the response (peak area of 4,5-naphthylpiazselenol expressed as AU). The calibration plot was obtained by linear least-squares regression.

The representative linear equation was $Y = (2.3 \pm 3.3) \times 10^5 + (66.4 \pm 0.9) \times 10^5 \times X$. The correlation coefficient (r) was close to unity (0.9991).

3.3.3. Limit of detection and quantitation

The limits of detection and quantitation (LOD: 0.102 μg ml^{-1} and LOQ: 0.309 μg ml^{-1}, respectively) were calculated in accordance with the 3.3 and 10 *s/m* criteria [36,37].

3.3.4. Solution stability

The stability of the solution of 4,5-naphthylpiazselenol in heptane after the derivatization was investigated. It was found that the derivative was stable for 5 h after its synthesis. However, it is advisable to prepare freshly standard and sample solutions prior to injection.

3.3.5. Accuracy

The accuracy of the proposed method was tested by:

(1) recovery experiment—analyzing reference standard solutions of known concentration; and
(2) comparing test results obtained in State Doping Control Laboratory (Kiev, Ukraine) by use of AAS method described in USP 29 for assay of selenium in oil- and water-soluble vitamins with minerals tablets [35b] using Shimadzu AA-6800 Atomic Absorption Spectrophotometer (Duisburg, Germany).

The results showing good recovery are summarized in Tables 2 and 3. When MLC and AAS results from assay of pharmaceutical formulations and premixes were compared (Table 3), statistical analysis using the Student's t-test and the variance ratio F-test showed that there were no significant differences between the results. The calculated t and F values were less than the theoretical values at 95% confidence level.

The influence of the matrix component was also investigated. According to literature [17] some cations and anions can influence the Se(IV) determination with 2,3-diaminonaphthalene and masking agent (EDTA or mixture of EDTA, sodium fluoride and sodium oxalate) should be added to the sample solution to increase the selectivity.

In this study, the effect of EDTA was investigated. According to the data of the manufacturer of Ferramin-vita syrup, it contains more than 100-fold quantities of cations (Fe(III), Zn(II), Ca(II), Mg(II), Cu(II), Mn(II), etc.) and 1000-fold quantities of anions (Cl^-, NO_3^-, SO_4^{2-}, asparaginate-ion, etc.) relative to the selenium content. It was found that EDTA has no significant effect on the selectivity and sensitivity of the analysis. The results are shown in Table 4. As it can be seen the recovery is good. From these results it can be concluded that the determination is not affected by the presence of the above-listed ions.

3.3.6. Precision

The precision as intra-day and inter-day reproducibility, expressed as R.S.D. %, was characterized by the spread of the data from replicate determinations.

Table 3
Results for determination of accuracy by corresponding test results from the new MLC method with those from an existing method

Dosage forms (DF)[a]	Found by proposed MLC method[b]		Found by AAS[b] (USP method [35a])		Δ[c] (%)	Calculated[d]	
	mg/DF	%	mg/DF	%		F-values	t-values
Ferramin-vita, syrup (1 μg mL^{-1} of Se as Na_2SeO_3)	1.08	108.23	1.09	109.22	0.99	4.85	2.11
Multi-tabs junior (50.0 μg/tablet of Se as H_2SeO_3)	50.7	101.42	50.3	100.60	−0.82	5.42	2.19
Beresh vitakid fizzy multi-vitamin tablets (10.0 μg/tablet of Se as H_2SeO_3)	9.55	95.53	9.65	96.48	0.95	5.11	2.27
Premixes for horses, pigs, cows (20.0 μg/g of Se as Na_2SeO_3)	21.83	109.14	21.95	109.71	0.57	4.88	2.01
Premixes for chicken and hens (50.0 μg/g of Se as Na_2SeO_3)	48.72	97.44	49.16	98.31	0.87	5.42	2.27

[a] Labelled concentration.
[b] Mean value from five determinations.
[c] Difference between the content obtained with respect to proposed method and that with respect to USP method.
[d] Tabulated t and F values for $P = 95\%$ are 2.31 and 6.39, respectively.

Table 5
Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for selenium (nominal concentration is 1.0 μg ml^{-1}) in a multi-vitamin syrup

Theoretical concentration (μg ml^{-1})	Intra-day[a]	Inter-day[a]			
	Mean ± R.S.D. (%)	Recovery amount ± R.S.D. (%)			Mean ± R.S.D. (%)
		Day 1	Day 2	Day 3	
0.795	100.2 ± 0.1	100.05 ± 0.09	100.3 ± 0.2	100.11 ± 0.06	100.2 ± 0.3
0.894	100.03 ± 0.08	99.98 ± 0.05	100.2 ± 0.3	99.9 ± 0.1	100.0 ± 0.4
0.993	100.2 ± 0.2	100.0 ± 0.3	99.95 ± 0.05	100.1 ± 0.1	100.1 ± 0.2
1.093	99.93 ± 0.09	99.98 ± 0.04	100.03 ± 0.08	99.95 ± 0.09	100.0 ± 0.1
1.192	99.7 ± 0.2	100.02 ± 0.07	99.88 ± 0.05	99.83 ± 0.08	99.9 ± 0.2

[a] Mean value of the five determinations.

For the intra-day reproducibility, i.e. repeatability, we performed 25 determinations covering the specified range. Reference standard solutions with selenium concentration of 0.795, 0.894, 0.993, 1.09 and 1.19 μg ml^{-1} were analyzed (five replicates each). Inter-day precision of the method was evaluated by analysis of freshly prepared selenium reference standard solutions on each of 3 different days under the same conditions.

The results are summarized in Table 5. These show that there was no significant difference between assay results either within-days or between days, indicating that the reproducibility of the method was good.

3.3.7. Robustness

The effect of different concentrations of organic modifier and SDS in the mobile phase, column temperature and flow rate was investigated. The chromatographic conditions are listed in Table 6. It is apparent that changing the concentration of 1-butanol and SDS in the mobile phase had no noticeable effect on the 4,5-naphthylpiazselenol chromatographic peak. Retention time slightly increased when the 1-butanol or SDS content was reduced. Changes in the column temperature or flow rate had similar effect.

On the basis of the data of the robustness test study, we proposed criteria for system suitability test: peak asymmetry and repeatability (R.S.D.) of retention time and peak area for replicate analyses. These are as follows:

- The asymmetry of the 4,5-naphthylpiazselenol peak, determined by analysis of the standard solution, should be not more than 1.8;
- The relative standard deviation of the 4,5-naphthylpiazselenol peak areas for five replicate injections of the standard solution should be not more than 2.0%.

3.4. Analysis of pharmaceutical preparations and premixes

The method developed has been applied for the determination of selenium(IV) in different multi-vitamin tablets, syrups and premixes. The pharmaceuticals (Ferramin-vita syrup, Multi-tabs junior and Beresh vitakid fizzy tablets) were commercially available in the Ukrainian market. Premixes samples were obtained from "Noris Ltd." (Kharkov, Ukraine).

A representative chromatogram of Ferramin-vita syrup is shown in Fig. 2d. It is evident that the peaks of excipients eluted

Table 6
Results of robustness test

Parameter	Variations	4,5-Naphthylpiazselenol		
		k	N × 10^{3}[a]	B/A
Mobile phase flow rate (ml min^{-1})	0.8	4.55	11.9	1.54
	1.0	4.53	12.4	1.38
	1.2	4.51	12.5	1.33
Column temperature (°C)	35	4.88	12.1	1.41
	40	4.53	12.4	1.38
	45	4.05	12.5	1.32
Detection wavelength (nm)	376	4.55	12.6	1.37
	378	4.53	12.4	1.38
	380	4.52	12.5	1.38
1-butanol volume fraction (SDS concentration is 0.05 mol l^{-1}, %)	9.5	4.82	10.9	1.44
	10.0	4.53	12.4	1.38
	10.5	4.32	12.6	1.34
SDS concentration (1-butanol volume fraction is 10.0%, mol l^{-1})	0.04	4.89	10.8	1.46
	0.05	4.53	12.4	1.38
	0.06	4.32	12.8	1.36

[a] Theoretical plates m^{-1}.

before 4 min to not interfere with the assay. The data in Table 3 show that the results of selenium(IV) determination in pharmaceuticals and premixes are in good agreement (96–110%) with the data declared by the manufacturers.

4. Conclusions

A new micellar liquid chromatographic (MLC) method for the determination of selenium(IV) contents in pharmaceutical syrups and tablets and premixes was developed. This can be an alternative to other methods—ICP-MS, AAS.

This method meets the requirements of "green chemistry" conception by using environment-friendly reagents. In addition, MLC technique allows excluding some sample preparation steps and reducing the cost of the mobile phase.

On the basis of the successful validation according to USP and ICH recommendations it can be stated that the proposed method is specific, linear, accurate and precise within the established range, and is therefore suitable for the determination of selenium(IV) in drugs and premixes.

References

[1] A. Berthod, M.C. Garcia-Alvarez-Coque, Micellar Liquid Chromatography, Marcel Dekker Inc., New York-Basel, 2000.
[2] H. Nishi, J. Chromatogr. A 780 (1997) 243–264.
[3] J. Esteve-Romero, S. Carda-Broch, M. Gil-Augusti, M.-E. Capella-Peiro, D. Bose, Trends Anal. Chem. 24 (2005) 75–91.
[4] E.M. Basova, V.M. Ivanov, O.A. Shpigun, Russ. Chem. Rev. 68 (1999) 1000–1083.
[5] T. Okada, J. Chromatogr. A 780 (1997) 343–360.
[6] A.U. Kulikov, A.G. Verushkin, L.P. Loginova, Chromatographia 61 (2005) 455–463.
[7] H. Ding, J. Wang, J.G. Dorsey, J.A. Caruso, J. Chromatogr. A 694 (1995) 425–431.
[8] M.G. Khaledi, Trends Anal. Chem. 7 (1988) 293–300.
[9] M. Albert, C. Demesmay, J.L. Rocca, Fresenius J. Anal. Chem. 351 (1995) 426–432.
[10] N. Lewen, S. Mathew, M. Schenkenberger, T. Raglione, J. Pharm. Biomed. Anal. 35 (2004) 739–752.
[11] C. B'Hymer, J.A. Caruso, J. Chromatogr. A 1114 (2006) 1–20.
[12] P. Suchocki, D. Jakoniuk, B.A. Fitak, J. Pharm. Biomed. Anal. 32 (2003) 1029–1036.
[13] H.K.Y. Lau, P.F. Lott, Talanta 18 (1971) 303–310.
[14] C. Sarzanini, E. Mentasti, J. Chromatogr. A 789 (1997) 301–321.
[15] G.B. Marshall, T.S. West, Anal. Chim. Acta 51 (1970) 179–190.
[16] R.F. Bayfield, L.F. Romalis, Anal. Biochem. 144 (1985) 569–576.
[17] P.F. Lott, P. Kukor, G. Moriber, J. Solga, Anal. Chem. 35 (1963) 1159–1163.
[18] B. Sun, M. Macka, P.R. Haddad, J. Chromatogr. A 1039 (2004) 201–208.
[19] A.-I. Stoica, G.-E. Babaua, E.-E. Iorgulescu, D. Marinescu, G.-E. Baiulescu, J. Pharm. Biomed. Anal. 30 (2002) 1425–1514.
[20] P.C. Uden, H.T. Boakye, C. Kahakachchi, J.F. Tyson, J. Chromatogr. A 1050 (2004) 85–93.
[21] M. Milano, A. Padro, R. Rubio, G. Rauret, J. Chromatogr. A 819 (1998) 211–220.
[22] B. Do, S. Robinet, F. Guyon, J. Chromatogr. A 918 (2001) 87–98.
[23] J.-F. Jen, Y.-J. Yang, C.-H. Cheng, J. Chromatogr. A 791 (1997) 357–360.
[24] V. Gergely, K.M. Kubachka, S. Mounicou, P. Fodor, J.A. Caruso, J. Chromatogr. A 1101 (2006) 94–102.
[25] S.V. Ketavarapu Yathavakilla, M. Shah, S. Mounicou, J.A. Caruso, J. Chromatogr. A 1100 (2005) 153–159.
[26] W. Goessler, D. Kuehnelt, C. Schlagenhaufen, K. Kalcher, M. Abegaz, K.J. Irgolic, J. Chromatogr. A 789 (1997) 233–345.
[27] L. Liang, S. Mo, P. Zhang, Y. Cai, S. Mou, G. Jiang, M. Wen, J. Chromatogr. A 1118 (2006) 139–143.
[28] A. Chatterjee, Y. Shibata, H. Tao, A. Tanaka, M. Morita, J. Chromatogr. A 1042 (2004) 99–106.
[29] A. Chatterjee, H. Tao, Y. Shibata, M. Morita, J. Chromatogr. A 997 (2003) 249–257.
[30] M. Hilp, J. Pharm. Biomed. Anal. 28 (2002) 337–343.
[31] J. Stray, J. Marek, K. Kratzer, F. Sebesta, Anal. Chim. Acta 57 (1971) 393–398.
[32] Y.J. Park, J.K. Hardy, J. Chromatogr. A 481 (1989) 287–297.
[33] A.F. Al-Attar, G. Nickless, J. Chromatogr. A 440 (1988) 333–352.
[34] A.U. Kulikov, L.P. Loginova, L.V. Samokhina, Chromatographia 57 (2003) 463–469.
[35a] United State Pharmacopoeia 29/National Formulary 24, USP Convention, Rockville, MD, 2006, pp. 3050–3053.
[35b] United State Pharmacopoeia 29/National Formulary 24, USP Convention, Rockville, MD, 2006, pp. 2425–2426.
[36] International Conference of Harmonization, Q2A: Text on validation of analytical procedures, US FDA Federal Register, V. 60, March, 1995, pp. 11260–11266.
[37] International Conference of Harmonization, Q2B: Validation of analytical procedures: methodology, US FDA Federal Register, V. 62, May, 1997, pp. 27463–27467.
[38] Y. Vander Heyden, M. Jimidar, E. Hund, N. Niemeijer, R. Peeters, J. Smeyers-Verbeke, L.D. Massart, J. Hoogmartens, J. Chromatogr. A 845 (1999) 145–154.
[39] G.A. Shabir, J. Chromatogr. A 987 (2003) 57–66.
[40] J.A. Adamovics, Chromatographic Analysis of Pharmaceuticals, Marcel Dekker, New York, 1997.