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Original Article

POLARIMETRY AND DYNAMIC LIGHT SCATTERING IN QUALITY CONTROL OF CARDIOTONIC AND HYPOTENSIVE TINCTURES

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ABSTRACT

Objective: To substantiate the possibility of using polarimetry to control the quality of tinctures as an additional pharmacopoeial method.

Methods: The polarimetric method (POL-1/2, Atago, Japan, the measurement accuracy of±0.002 °) was used to measure the optical activity (α °) of motherwort, valerian and hawthorn tinctures. The dynamic light scattering method (DLS; Zetasizer Nano ZS, Malvern, UK) was used to assess the stability of alcoholic and aqueous dilutions of tinctures according to the intensity of dynamic light scattering dependent on the size (d, nm) of the dispersed phase particles and the values of the electrokinetic potential (ξ, mV).

Results: For the first time in this investigation, the polarimetry approach was proposed to evaluate the cardiotonic and hypotensive tinctures' quality and for their identification. Valerian tincture, dilution 1:40,-0.10°<α°<-0.89°; motherwort, tincture-dilution 1:10,-0.10°<α°<-2.21°; hawthorn, tincture without dilution,-0.76°<α°<-1.55°-these are the acceptable ranges of optical activity (α°) of their alcohol dilutions. Beyond these intervals, the use of the polarimetric approach is impossible. Values of optical activity below 0.1 correspond to too low a content of optically active components. Tinctures with optical activity above the upper value of the interval were unstable dispersed systems with low values of the electrokinetic potential (|ξ|≪25mV) and micron particle sizes. Reference tinctures were made from raw materials (*Leonurus cardiaca* L.) to verify the results. The quality parameters: optical activity (α°), spectra of dynamic light scattering by intensity, volume, and number ("I-d"; "V-d"; "N-d"), electrokinetic potential (ξ) values, and photon pulse count per second (Count Rate, kcps) corresponded to the results obtained for pharmaceutical dosage forms.

Conclusion: The permissible intervals of optical activity (α°) of their ethanol dilutions, as well as their relationships with the particle size of the dispersed phase and the values of the electrokinetic potential, were established for the first time to evaluate the quality of tinctures. The obtained results show that polarimetry can be recommended as an additional pharmacopoeial quality control method for tinctures.

Keywords: Cardiotonic and hypotensive tinctures, Optical activity, Polarimetry, Dynamic light scattering, Electrokinetic potential

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INTRODUCTION

Tinctures of motherwort, valerian, and hawthorn are frequently used as medications due to their sedative, hypotensive, cardiotonic, and other effects. Contradictory findings from clinical research exist, which may be the result of lax quality control standards for pharmaceutical dosage forms. [1-4]. The search for new methods of assessing the quality of tinctures is necessary due to the shortage or high cost of chemical reference substances. In addition, there are no pharmacopoeial monographs for most individual tinctures and a section on quality control in the general monograph "Tinctures" [5]. Unfortunately, the quality control of tinctures is mainly evaluated by the amount of dry residue after the removal of solvents. Only valerian tincture has undergone the most extensive research, and chemical reference substances have been created for its analysis using the HPLC method [2, 6]. From the available scientific literature of the last decade, attention is drawn to the quality control of propolis tincture [7], where the total amount of flavonoids was determined by UV/Vis spectrophotometry and 14 of them were identified using HPLC coupled to a Dioderray Detector (HPLC/DAD). As can be seen, physicochemical methods must be used to improve the quality control of tinctures and other herbal extracts [8–12]. Polarimetry has never been used in these studies.

We proposed the use of the polarimetry method to evaluate the alcoholic extract quality of plant raw materials, taking into consideration the existence of optically active compounds in plants. The optical rotation angle ranges of the following tincture dilutions were established: motherwort tincture, dilution 1:10,-0.10°<α°<-2.21°; valerian tincture, dilution 1:40,-0.10°<α°<-0.89°; and hawthorn tincture, without dilution,-0.76°<α°<-1.55°. Beyond these intervals, the use of the polarimetric approach is impossible. Values of optical activity below 0.1 correspond to too low a content of optically active components. Tinctures with optical activity above the upper value of the interval were unstable dispersed systems with low values of the electrokinetic potential (|ξ|≪25mV) and micron particle sizes.

This study aims to support the possibility of polarimetry as a potential alternative pharmacopoeial tool for quality control of tinctures.

MATERIALS AND METHODS

Pharmaceutical dosage forms

Motherwort, valerian, and hawthorn tinctures from different manufacturers (n≥25 for each tincture) were obtained from pharmacies. were purchased from pharmacies. Motherwort herb was provided by the company "PharmaTsvet".

Solvents

Ethanol, 96% (v/v) for pharmaceutical production and analysis, Merck Emprove®Expert, and high-resistance purified water (Milli-Q®Integral unit) were used for dilution of tinctures.

Preparation of the reference sample

Reference motherwort tinctures were prepared from raw materials (*Leonuri herba*) [5]. The raw materials were dispersed by dry grinding in a cutting mill (acc. to Rumpf) under the control of the particle size (<1-2 mm) by optical microscopy (Altami BIO2). The raw materials were infused in 70% ethanol at the ratio of 1:5 and the temperature of 20 °C for 7 d with stirring. After infusion, the solution was separated from the raw material by decantation, the

remaining raw material was squeezed out, washed with a new portion of the solvent, squeezed out again and brought to the required volume. The tinctures were left for 4 d at 8 °C. The tincture was passed through filters with the pore size of 2-3 μm and stored in a cool, dark place in a sealed container.

Polarimetry

Optical activity of tinctures and their dilutions was determined using the Atago POL-1/2 polarimeter (Japan), in a 100 mm cell, the measurement accuracy of±0.002° and the resolution of 0.0001°. The electronic Peltier module was used for setting the required temperature (T=20 °C).

Dynamic laser light scattering (DLS)

The measurements of the particles size (d) of the dispersed phase in the nano-and micrometer ranges and the electrokinetic potential (ξ) were carried out by dynamic laser light scattering (DLS) (Zetasizer Nano ZS, Malvern, UK).

Statistics

The results were processed using software packages Origin Pro 9.1 (OriginLab, USA). Each value on the fig. represents «mean±SD» or average±confidence interval ($\bar{x}\pm\Delta\bar{x}$; P=0.05).

RESULTS AND DISCUSSION

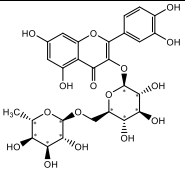
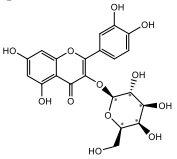
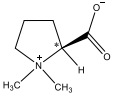
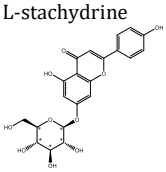
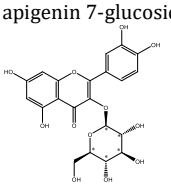
The literature provides significant scientific data on the study of the chemical composition of motherwort herb (*Leonurus cardiaca* L.) [1, 13, 14], root/rhizome of valerian (*Valeriana officinalis L.*) [2], fruit, leaves with flowers of hawthorn *(Crataegus genus)* [3]. For example, the structures of about 300 substances have been isolated and determined from the motherwort *(Leonurus genus)* [13, 14]. They are represented by alkaloids, monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, iridoids, flavonoids, phenylpropanoids, steroids, cyclic peptides, etc. But so far, the chirality of these compounds and the possibility of quality control of their alcoholic extracts by the polarimetric method have not been discussed in the scientific literature.

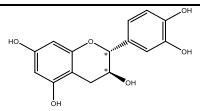
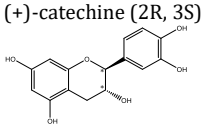
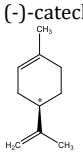
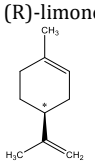
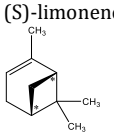
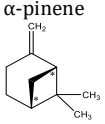
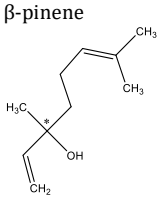
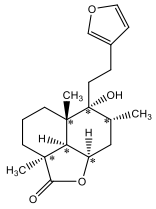
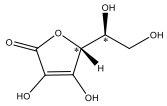
The majority of physiologically active substances found in medicinal plant materials have one or more chiral centers that make them optically active. As an illustration, there are at least 12 such compounds in motherwort raw materials (table 1). As water-alcohol extracts, tinctures are required to have optical activity, the sign and magnitude of which depend on the chemical composition, content of chiral compounds, and kind of solvent. The optical activity of motherwort, valerian, and hawthorn tinctures was validated by polarimetric measurements of alcohol dilutions of these tinctures. Regardless of who made the end dosage form, the rotational angles of the polarized light plane had negative values.

Since the rise in the angle of optical rotation was noticed over time, the optical activity for dilutions of 1:10 of the motherwort tincture was measured immediately after preparation (fig. 1). It is very likely connected to chiral switching [15]. An acceptable range of values for the optical rotation angle for the samples under investigation was determined to be between-0.10° and-2.21° (n=13, $\bar{x}\pm\Delta\bar{x}$=−0.616±0.381). Values |α°| less than 0.10 ° corresponded to solutions containing very few optically active compounds and were thus excluded from consideration. These solutions were almost colorless, which also testified to the low quality of tinctures. The low optical activity could be due to the use of low-quality raw materials or an insufficient degree of extraction of biologically active chiral components.

Tinctures with optical activity above the upper value (|α°|≥2.21°) of the interval were unstable dispersed systems with low values of the electrokinetic potential (|ξ|≪25mV), because of the presence of large unstable particles of the dispersed phase with sizes matching the micrometer range [10, 16]. These particles interfered with polarimetric measurements, and they were also excluded from consideration.

Table 1: Optically active chemical compounds contained in motherwort herb (*Leonuri herba*)

No	Optically active compound	Specific rotation angle, $[\alpha]^{20}_D$ (concentration, solvent)	References
1	 quercetin 3-rutinoside **(**rutin)	+13.82 (1%, ethanol)	[17]
2	 hyperin (hyperoside)	-83 (0.2%, pyridine)	[17]
3	 L-stachydrine	-40.25 (4%, water),	[17]
4	 apigenin 7-glucoside (cosmosiin)	-64 (pyridine)	[17]
5	 isoquercetin	$[\alpha]^{25}_D$-19.5 (0.47% pyridine)	[17]

6	 (+)-catechine (2R, 3S)  (-)-catechine (2S, 3R)	+17; $[\alpha]^{18}_{D}$ =+16 to+18.4° -16.8	[18]
7	 (R)-limonene  (S)-limonene	+126.8 -122.6	[17]
8	 α-pinene	(-)-α-pinene [a]$^{23}_{D}$-51.5 (neat) (99.8% ee) (+)-α-pinene [a]$^{23}_{D}$+51.4 (neat) (99.6% ee)	[17]
9	 β-pinene	(-)-β-pinene [a][23]$_{D}$-22.8 (neat) (100% op) (+)-β-pinene +28.6	[17, 19]
10	 linalool	L-Linalool (-17) D-Linalool (+19)	[17, 19]
11	 marrubiin	+35.8° (3.1%, chloroform); [a]^{24} $_{D+}$45° (acetone)	[18]
12	 ascorbic acid	[a]$^{25}_{D}$ from+20.5 to+21.5° (1%, water); [a]$^{23}_{D}$+48° (1%, methanol)	[18, 19]

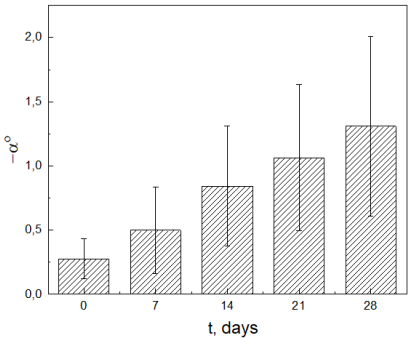


Fig. 1: Increase in optical activity of motherwort tinctures (1:10) over time (n=9, $\bar{x}\pm\Delta\bar{x}$)

The value of the potential, which was in the range of-(14÷17) mV, was used to assess the stability of freshly prepared alcohol solutions. As the angle of optical rotation increased, the value of the electro kinetic potential decreased with time and reached-(4÷6) mV on the 28th day.

The study of alcohol and water dilutions showed that the latter had greater stability. Water dilutions had electro kinetic potentials higher than the limiting values of the stability of the colloidal system (25 mV) [10, 16] and reached-34 mV. It was reasonable to assume that stable nanoparticles in aqueous dilutions would freely penetrate cells (for example, due to pinocytosis) and transport biologically active substances. The size spectra of these particles have a maximum at about 150 nm (fig. 2). Alcoholic solutions differed in the presence of significantly larger micrometer-sized particles at the same dilutions.

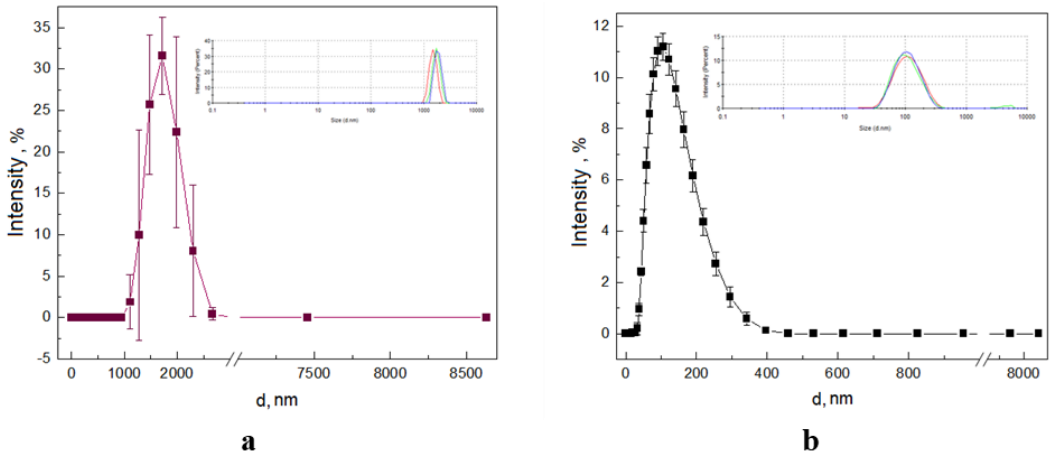


Fig. 2: Size spectra of dynamic light scattering (DLS) of freshly prepared alcohol (a) and water (b) dilutions of motherwort tinctures (1:20). Data is given as mean±SD, n=3

To confirm the correctness of the chosen interval of the optical rotation angle, reference tinctures of motherwort were prepared. By parallel infusion, 3 motherwort tinctures were obtained, dilutions of which with 96% ethanol in a ratio of 1:10 had the following values of optical rotation angles:

$\bar{\alpha}$=-1.2276°±0.4738° (α_1=-1.3110°, α_2=-0.7176°, α_3=-1.6542°). Thus, the optical activity of the solutions corresponded to the previously established range of α ° values for high-quality pharmaceutical dosage forms.

The reference tinctures under investigation have been characterized by the intensity, volumetric and numerical distribution of particles of the dispersed phase (fig. 3). As can be seen, the light scattering intensity and volumetric distribution correspond to particles with the maximum at 5 μm. However, the numerical distribution is more informative, showing that there are smaller particles in the nanometer range, with the maximum of the distribution occurring at 500 nm. The values of ξ-potential and photon pulse count per second, which were-(14.24÷16.5) mV and (210-480) kcps, respectively, did not exceed the values stipulated for pharmaceutical dosage forms.

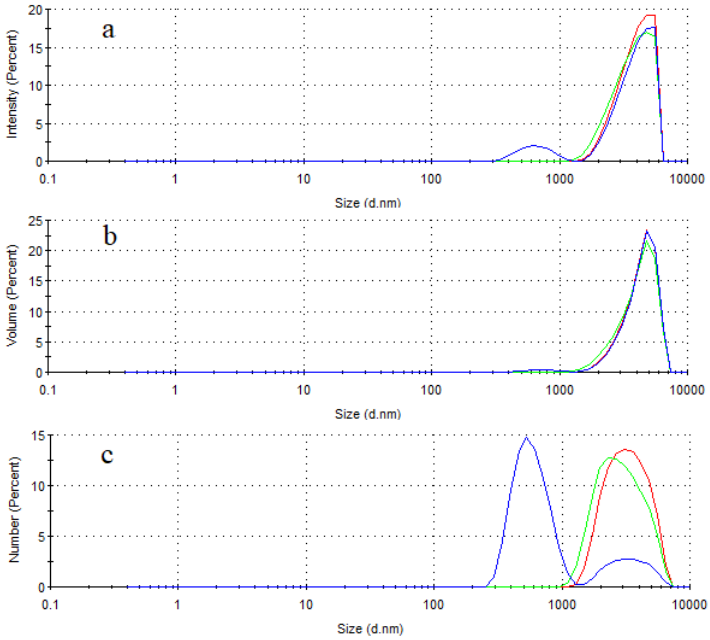


Fig. 3: Size distribution of light scattering intensity (a), volume (b) and number of particles (c) of the dispersed phase of alcohol dilutions (1:20) of reference tinctures

In the example of hawthorn tincture, the stability of the colloidal system was evaluated in the form of the dependence of the electrokinetic potential on the angle of optical rotation (fig. 4). As can be seen, an increase in the content of optically active components, ξ-potential decreases. This confirms the need for the upper limit of the selected interval of the α° values. As expected, the number of photon pulses per second (kcps) increases with α °, occupying the recommended range of 100 to 500 kcps per second. But at low values of the angle of rotation (|α°|<0.5°), the linear dependence was violated, and on the chosen scale, all points were grouped in the range of 150-200 kcps. It is likely that in solutions with a low content of optically active compounds, the contribution of giant heterogeneous water clusters to the optical activity increases [12, 20].

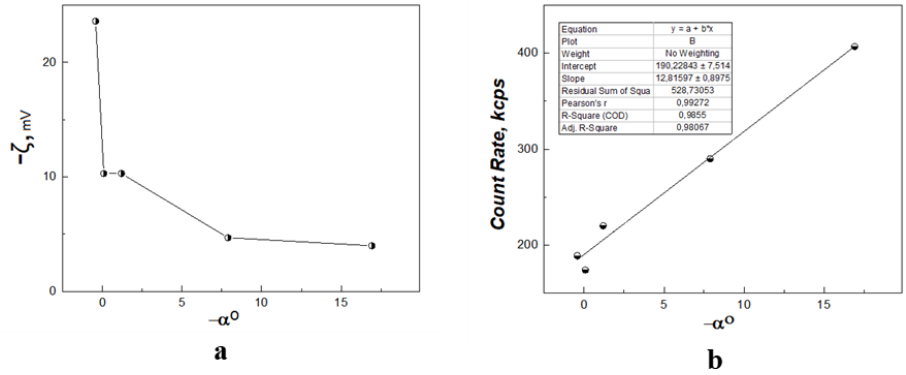


Fig. 4: Correlation of optical activity α° with ξ-potential (a) and number of photon pulses (b) for hawthorn tincture

The results obtained make it possible not only to evaluate the quality but also to identify the tinctures. To do this, it is necessary to follow the sequence of actions:

-Dilute tinctures of valerian, motherwort, and hawthorn with 96% ethanol in a ratio of 1:40 so that the volume corresponds to the size of the polarimeter cuvette. The angle of optical rotation of valerian tincture is in the range −0.10°<α°<−0.89° (n=10, $\bar{x}\pm\Delta\bar{x}$ =-0.240±0.093). The α° values of motherwort tincture and hawthorn tincture in the indicated dilution are close to zero.

-Dilute motherwort and hawthorn tinctures with 96% ethanol in a ratio of 1:10. The angle of optical rotation of motherwort tincture is in the range −0.10°<α°<−2.21° (n=13, $\bar{x}\pm\Delta\bar{x}$=-0.616±0.381). The α° value of hawthorn tincture in the indicated dilution is close to zero.

-Measure the optical activity of hawthorn tincture without dilution. The angle of optical rotation is in the range-0.76°<α°<−1.55° (n=7, $\bar{x}\pm\Delta\bar{x}$=-1.17±0.27).

The results show that valerian tincture has the highest concentration of optically active substances, and hawthorn tincture contains the least amount of chiral components and therefore does not require dilution for polarimetric studies.

Thus, the polarimetric method can be used to control the quality and determine the authenticity of tinctures, which is explained by the presence of chiral compounds extracted from plant materials.

Without a doubt, the polarimetric evaluation of tinctures from other pharmacological classes, including those with antibacterial and anti-inflammatory activities, is of interest [7, 21].

CONCLUSION

In this study, for the first time, the polarimetry method was used to assess the quality and identify cardiotonic and hypotensive tinctures. Permissible ranges of optical activity (α°) of alcohol dilutions for the tinctures were determined. The optical activity of the prepared reference motherwort herb tinctures confirmed the precision of the quality intervals and their relationship to the stability parameters: particle size spectra in the nano-and micrometer ranges, as well as electrokinetic potentials. The obtained results prove that polarimetry can be recommended as an additional method of pharmacopoeial quality control of tinctures.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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