Numerical Indicators of Absorption Spectra in Herbal Formulation Quality Assessment

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Abstract

The study is dedicated to absorption spectrophotometry of herbal formulations. The objective is to develop the methodology for determining numerical indicators of the turning points, steps and maximums of the absorption spectrum (AS) lines to evaluate the quality of herbal extractions. Ultraviolet absorption spectrophotometry of the extractions was prepared in different conditions from garden sage leaves and hawthorn berries. AS have been processed with proprietary software calculating the wavelengths, optical densities, first derivatives in the turning points, steps, maximums and rates of the absorption bands. For the purposes of statistical processing Student's t-test and Z-Sign test have been applied. When the conditions for preparing the extractions were changed, the variations of values of the derivatives in the turning points and steps proved to be most evident and the changes of values of optical densities and wavelengths in AS maximums were less expressed. Significant differences from the standard AS numerical indicators were observed in cases when the extractions were prepared under varying conditions or with substandard raw material quality giving grounds for rejecting the extracts under investigation.

Due to the sufficient pharmaceutical precision, low associated costs and the possibility to obtain the results quickly, it seems advisable that the numerical indicators under investigation should be used for the purpose of spectrophotometric fast analysis in the course of evaluating the quality and authenticity of herbal formulations. The aggregate of the obtained numerical indicators represents a spectrophotometric passport of the extraction and can be included in the regulatory documents for pharmaceutical drugs of plant origin.

Keywords: Sage, Hawthorn, Extractions, Quality Control, Applied Spectrophotometry.

Introduction

In spectrophotometric pharmacopoeia analysis of pharmaceutical drugs[1,17] and plant extractions,[2,3,7] the principal numerical indicators are wavelength (*lm*) and optical density (*Am*) of the maximum of absorption spectrum (AS) and the auxiliary indicators are half-breadth of the absorption band (AB), integrated intensity (*S*), asymmetry and others.[8] Traditionally, to determine additional numerical indicators (ANI) a researcher, as a rule, takes the wavelengths of the points of "half level" 0.5*Am* of AS line which can be done easily with the bell-shaped line (Gaussian curves).

In particular, AS line of the tincture prepared from the bark of the black birch (*Betula mandshurica* (Regel) Nakai, species Betulaceae) at the level of 0.5*Am* has two points *a* and *b* with abscissas λ_a and λ_b (Figure 1, Curve 1). The half-width of AB corresponds to the difference $\lambda_b - \lambda_a$ and the absorption intensity *S* of the area under AS line is within the range of the specified wavelengths.[8] In as much as the shape of the line of AS band depends on the conditions of the extraction, on the quality of the raw materials[5], on solvent[6] and on other factors, then, obviously, ANI that reflects the specific features of AB bands can also be used to characterize the extracts.

However, the use of traditional ANI is complicated by the fact that the lines of AS bands of plant extractions differ considerably from Gaussian distribution. For example, AS line of the tincture prepared from motherwort leaves (*Leonurus cardiata* L., species Lamiaceae) has only one point at the half level (point *c* on Curve 2, Figure 1); therefore, in this case ANI cannot be determined.

Thus, the use of the points of "half level" to define traditional ANI in spectrophotometric analysis of herbal formulations is limited. Besides, traditional ANI can by no means be used to obtain numerical characteristics of the steps that often change in AS lines (e.g. Figure 3) or to describe the monotone AS[8] which requires selecting other reference points of AS line. The turning points of AB line are, probably, the most suitable for the purpose; however, the use of such points for pharmaceutical spectrophotometric analysis of herbal extractions has not been sufficiently investigated. The objective of this study is to develop the methodology for determining the numerical indicators of the turning points, steps and maximums of AS lines to evaluate the quality of herbal extractions.

Material and Methods

For the purposes of investigation, the samples are represented by the commercial specimens of pharmaceutical herbal formulations: garden sage leaves (*Salvia officinalis* L., species Labiatae) in which tincture is applied as local anti-inflammatory agent due to its antioxidant properties[11,15,18] berries of redhaw hawthorn (*Crataegus sanguine* Pall., species Rosaceae), Chinese hawthorn (*Crataegus pinnatifida* Bunge.) which tinctures with 70 % ethanol are applied in cases of cardiac functional illness.[4,13]

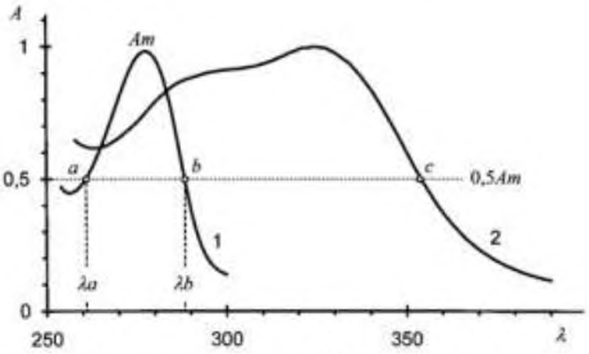

Fig. 1: Absorption spectra of tinctures prepared from the bark of Manchurian birch (1) and from quinquelobate motherwort leaves (2). ***Am*** **– absorption maximum,** ***a*** **and** ***b*** **– points of "half level",** ***λa*** **and** ***λb*** **– the wavelengths corresponding to these points. Vertical direction – optical density** ***A*** **in nominal units, horizontal direction – wavelength** ***λ*****, nm.**

In the 1st series of experiments, the tincture was prepared from the sage leaves in standard conditions ("Control-1") according to the requirements of Pharmacopeia (Pharmacopoeia of the USSR, 1990): 10 g of sage leaves manufactured by CJSC "Ivan-Chay" were put into enameled cookware, poured with 200 ml of boiling water and with the lid on, were held in boiling-water bath for 15 min; then the tincture was cooled at indoor temperature within 45 min, filtered, pressed and refilled with boiled water to restore the initial volume. For the purposes of comparison, the sage leave tinctures were also prepared with different deviations from the standard conditions: extraction with cold water without bath; using the raw materials whose shelf-life expired, extraction with spirit: 10 g of leaves were poured with 200 ml of 95 % ethanol, infused for three days, filtered, pressed, refilled with spirit up to the initial volume (Pharmacopoeia of the USSR, 1990).

In the 2nd series of experiments, the standard tincture ("Control-2") was prepared from hawthorn berries: 10 g of redhaw hawthorn berries were mixed with 70 % ethanol in the amount sufficient to obtain 100 ml of tincture, infused for 3 days, filtered, pressed, refilled with 70 % ethanol to restore the initial volume (Pharmacopoeia of the USSR, 1990). For the purposes of comparison, the extractions were also prepared with different deviations from the standard conditions: extraction with 70 % and 40 % ethanol or water, infusing Chinese hawthorn with 70 % ethanol. Besides, commercial pharmaceutical drug "Hawthorn tincture" manufactured by PJSC "Flora Kavkaza" was used.

AS of the extractions were registered with digital spectrophotometer UV-2501PC (Shimadzu, Japan), with incremental step of 1 nm in the range of 230 – 450 nm. In each option of the experiments 15 spectra were registered. The spectra were processed by proprietary software[9] (No. 2009614442 in the State Registry of Intellectual Property). The program envisages the algorithms of numerical differentiation and calculation of special points taking into account the fact that the first-order derivative in the maximum of the function changes its sign from "+" to "–"; in turning points it reaches the maximal and in step points it reaches the minimal values in their absolute magnitude (Figure 1 and 2, Curves *Sp*, the scales of the derivatives along the vertical axis are reduced to the scales Abs for visualization purposes).

Assisted by the abovementioned program, the authors of this study performed normalization of AS for the highest maximum, calculated the wavelengths *λ*, optical densities *A*, values of derivatives (*df*) in turning points and in the steps of AB line and also the intensity *S* of absorption within the range of the turning point wavelengths that are closest to the maximum on the left and on the right.

In the course of statistical processing, Student's t-test for small sample and Z-Sign test have been applied.[10]

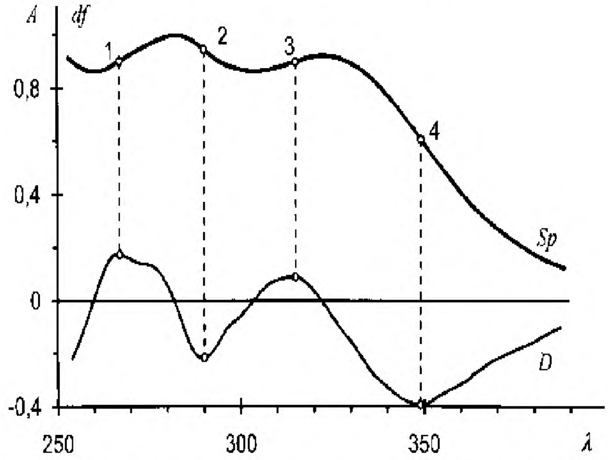

Figure 2: Absorption spectrum of sage leaves tincture (*Sp*) and its first-order derivative (*D*). 1, 2, 3 and 4 – turning points. Vertical direction – optical density *A* and values *df* of the first derivative in nominal units, scale of *df* is reduced to scale of *A*. Horizontal direction – wavelength *λ*, nm.

Results

The obtained results show that AS of the standard sage tincture ("Control-1") is characterized by two maximums of absorption in UV range (Figure 2) and by four turning points with their wavelengths *λ*, optical densities *A* and values of the first derivatives *df* and also by the intensities *S* of band absorption that make 18 numerical indicators in total (Table 1). Optical density *Am* of the second maximum is by 7.5 % lower than the first one but the intensity *S* of absorption of the second band is by 12 % higher ($p < 0.05$) than that of the first one.

Table 1
Numerical indicators of AS for garden sage leaves extractions prepared under different conditions

Band No.	**Indicators**			**Options of experiments**			
				Standard ("Control-1")	**Extraction with cold water and without bath heating**	**Expired raw materials**	**Extraction with 95 % ethanol**
1	λm			283 ±1	281 ±1 (-)	285 ±1 (-)	289 ±1* (+)
	Am			1 ±0.01	1 ±0.01 (0)	0.956 ±0.081* (+)	0.819 ±0.082* (+)
	Turning points	left	λ	267 ±1	274 ±1* (+)	277 ±1* (+)	269 ±1* (+)
			A	0.902 ±0.019	0.966 ±0.021* (+)	0.890 ±0.068 (-)	0.529 ±0.053* (+)
			df	0.0093 ±0.0004	0.0060 ±0.0006* (+)	0.0135 ±0.0015* (+)	0.0153 ±0.0014* (+)
		right	λ	293 ±1	289 ±1* (+)	290 ±1* (+)	293 ±1 (-)
			A	0.912 ±0.01	0.942 ±0.002* (+)	0.934 ±0.045 (-)	0.801 ±0.042* (+)
			df	-0.0094 ±0.0002	-0.0134 ±0.0007* (+)	-0.0071 ±0.0006* (+)	-0.0069 ±0.0007* (+)
	S			25.9 ±1.5	15.7 ±1.6* (+)	13.1 ±0.8* (+)	13.6 ±1.1* (+)
2	λm			323 ±1	320 ±1* (+)	324 ±1 (-)	329 ±1* (+)
	Am			0.925 ±0.012	0.869 ±0.016* (+)	1 ±0.01* (+)	1 ±0.01* (+)
	Turning points	left	λ	315 ±1	312 ±1* (+)	311 ±1* (+)	318 ±1* (+)
			A	0.899 ±0.013	0.848 ±0.019* (+)	0.925 ±0.014 (-)	0.898 ±0.076 (-)
			df	0.0063 ±0.0003	0.0053 ±0.0003* (+)	0.0078 ±0.0006* (+)	0.0207 ±0.0031* (+)
		right	λ	349 ±1	345 ±1* (+)	350 ±1 (-)	345 ±1* (+)
			A	0.608 ±0.013	0.642 ±0.006* (+)	0.610 ±0.051 (-)	0.742 ±0.058* (+)
			df	-0.0166 ±0.0002	-0.0174 ±0.0013 (-)	-0.0247 ±0.0004* (+)	-0.0310 ±0.0029* (+)
	S			29.1 ±1.1	25.8 ±0.9* (+)	32.9 ±1.4* (+)	25.6 ±1.2* (+)

Note: * – significant difference from "Control-1" at $p < 0.05$, sign symbols in brackets.

AS line of the extractions prepared under the conditions that are different from the standard conditions also has two maximums and the numerical indicators to that or another extent, deviate from "Control-1". The largest number, 16 of 18, of significant differences was obtained for AS of the sage tincture with 95 % ethanol; for AS of the tincture prepared with cold water and without bath heating these deviations made 15 of 17 and for the tincture with the expired raw materials, this number amounted to 11 of 18 which in terms of Z-criterion corresponds to significance level p of zero hypothesis in the range of 0.01 to 0.05.

Thereat, the differences of numerical indicators of AS of non-standard extractions from those of "Control-1" have reached the figures as follows: absorption intensity 23 – 98 %, derivatives 14 – 80 % and optical densities 6 – 21 % in almost all turning points. For the left turning point in the first band of AS of the tincture with 95 % ethanol, the difference amounts to 71 %. For the first AB, the optical densities of the maximums Am are differing from "Control-1" by 4 – 21 % only for AS of the tinctures that were prepared with the expired raw materials and for AS of the tincture prepared with 95 % ethanol, but for the second AB, this difference makes just 6 – 8 %. The wavelengths λm of the maximums of absorption differ from "Control-1" by 2 – 6 nm.

Registered AS of the standard hawthorn tincture ("Control-2") was characterized by single maximum in UV range (Figure 3), by one turning point on the left and two turning points on the right and by the step on the right slope of AS line with its wavelengths, optical densities and derivatives that make 15 numerical indicators in all (Table 2).

Among the numerical indicators of AS of the commercial sample of "Hawthorn tincture" and of Chinese hawthorn berry tincture, just one significant difference has been observed from "Control-2" of 12 potentially possible ones that according to Z-criterion, it proves the consistency of zero hypothesis. The obtained result is quite expectable: commercial specimen manufactured by JSC "Flora Kavkaza", obviously, meets the standard requirements and Chinese hawthorn is close to the hawthorn species that are traditional for medical science.

The number of significant deviations from "Control-2" of the numerical indicators of AS of the extractions with 40 % ethanol or water amounted to 8 of 14 and 9 of 11 (Table 2) in terms of Z-criterion corresponds to significance level p of zero hypothesis lower than 0.05.

Table 2
Numerical indicators of AS for hawthorn berries extractions prepared under different conditions

Indicators				**Experiment options**				
				Hawthorn species				**Tincture manufactured by JSC "Flora Kavkaza"**
				Redhaw hawthorn			**Chinese hawthorn with 70 % ethanol**	
				Standard ("Control-2")	**tincture with 40 % ethanol**	**Aqueous infusion**		
Maximum	λm			280 ±1	276 ±1* (+)	273 ±1* (+)	280 ±1 (0)	280 ±1 (0)
	Am			1 ±0.01	1 ±0.01 (0)	1 ±0.01 (0)	1 ±0.01 (0)	1 ±0.01 (0)
Turning points	left		λ	269 ±1	268 ±1 (-)	267 ±1* (+)	270 ±1 (-)	269 ±1 (0)
			A	0.838 ±0.067	0.966 ±0.056* (+)	0.985 ±0.011* (+)	0.853 ±0.061 (-)	0.795 ±0.063 (-)
			df	0.0223 ±0.0019	0.0068 ±0.0007* (+)	0.0045 ±0.0004* (+)	0.0218 ±0.0016 (-)	0.0263 ±0.0262 (-)
	right	1st	λ	289 ±1	288 ±1 (-)	289 ±1 (0)	289 ±1 (0)	290 ±1 (-)
			A	0.746 ±0.056	0.816 ±0.079 (-)	0.842 ±0.044 (-)	0.739 ±0.061 (-)	0.754 ±0.048 (-)
			df	-0.0489 ±0.0032	-0.0320 ±0.0029* (+)	-0.0241 ±0.0008* (+)	-0.0485 ±0.0034 (-)	-0.0463 ±0.0036 (-)
		2nd	λ	336 ±1	337 ±1 (-)	336 ±1 (0)	333 ±1* (+)	335 ±1 (-)
			A	0.317 ±0.029	0.360 ±0.031* (+)	0.439 ±0.032* (+)	0.306 ±0.024 (-)	0.334 ±0.032 (-)
			df	-0.0059 ±0.0004	-0.0043 ±0.0006* (+)	-0.0064 ±0.0002* (+)	-0.0062 ±0.0006 (-)	-0.0083 ±0.0006* (+)
Step	λ			307 ±1	304 ±1 (-)	307 ±1 (0)	309 ±1 (-)	308 ±1 (-)
	A			0.449 ±0.041	0.553 ±0.026* (+)	0.628 ±0.048* (+)	0.429 ±0.016 (-)	0.473 ±0.042 (-)
	df			-0.0026 ±0.0003	-0.0079 ±0.0008* (+)	0.0072 ±0.0004* (+)	-0.0028 ±0.0003 (-)	-0.0020 ±0.0002 (-)
S				19.3 ±1.1	20.1 ±1.1 (-)	22.6 ±1.2* (+)	18.4 ±1.1 (-)	20.0 ±1.4 (-)

Note: * – significant difference from "Control-2" at $p < 0.05$, sign symbols in brackets.

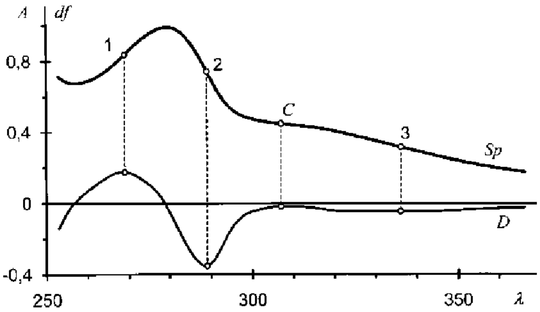

Figure 3: Absorption spectrum (*Sp*) of redhaw hawthorn tincture and its first-order derivative (*D*). 1, 2 and 3 – turning points, *C* – step. For other indicators see figure 2.

The values of the derivatives differ from "Control-2" in the left turning points 3.28 – 4.95 times and in the points of steps 2.77 – 3.03 times. The deviations from "Control-2" of the significant values of optical densities are less expressed: in the left turning points 1.15 – 1.17 times, in the second right 1.13 – 1.38 times and in the step 1.23 – 1.40 times. The differences of optical densities of the maximums and of the 1st right turning point from "Control-2" are not significant and the wavelengths of the maximums, of the turning points and steps differ from "Control-2" by just 1 – 3 nm.

Findings

The obtained data show that the numerical indicators of AS of the extractions that were prepared under non-standard conditions (sage leaves extraction with cold water instead of hot, using the raw materials which shelf-life expired, variations of ethanol concentrations, Tables 1 and 2) are statistically significantly different from the standard. And on the contrary, under the conditions of extract preparation that are close to the standard ones (tincture with Chinese hawthorn berries or commercial hawthorn tincture manufactured by "Flora Kavkaza"), the numerical indicators of AS of the extractions statistically coincide with the standard. Thus, in terms of the numerical indicators of AS, it is possible to judge on the deviations from the standard values which were observed in the extractions that were prepared violating the technological regulations and this gives the grounds to reject them.

The most serious significant differences from "Control" have been observed with such numerical indicators based on the turning points of AS line as absorption intensity, value of the derivatives in the turning points and the steepness of the steps. Thereat, the differences of the traditional numerical indicators of the wavelengths and optical densities of the maximums of AS from "Control" are statistically insufficiently significant. The ANI that have been used by the authors of this study and that are based on the turning points of AS line are most significant and it seems advisable that they should be used for further characterization of extraction properties.

It takes 5-7 min to obtain the numerical indicators for one AS including spectrum registration and software processing; thereat, no preliminary treatment of samples or any additional reagents are required except the extraction solvent and this represents the advantage of the spectrophotometric method described above as compared to liquid chromatography[16]. The accuracy of the digital spectrophotometer of circa ±0.004Abs (Research class spectrophotometers, 2016) is wholly satisfactory for pharmaceutical purposes.

Also, it should be noted that the study made use of the numerical indicators of normalized AS that do not depend on the concentrations of the extractions which is of no small importance in the practices of spectrophotometry of herbal formulations, in as much as it is very difficult to standardize their precise concentration. In all, the results of the undertaken investigations demonstrate wider opportunities for molecular spectrophotometry in the analysis of herbal formulations applying the turning points of AS line instead of the traditional "half level" points and this is important for further development of spectrophotometric method.

Conclusion

Under changed conditions of extraction preparation, the variations of the values of the derivatives in the turning points and in the steps, are most evident but the changes of the values of optical densities and wavelengths in AS maximum points are less expressed. Significant deviations from the standard of the numerical indicators of AS were observed in cases when the requirements to the conditions of the extraction preparation are not met or when the quality of the raw materials is improper. They can serve as the reason for rejecting the extractions under investigation. Given sufficient pharmaceutical precision, low associated costs and the possibility to obtain the results quickly, it seems reasonable that the investigated numerical indicators should be used in spectrophotometric fast analysis of herbal formulations.

The aggregate of the obtained numerical indicators represents a spectrophotometric passport of the extraction and can be included into the regulatory documentation for pharmaceutical drugs of herbal origin.

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