Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Formulation, stability and analytical method validations of combined St. John's wort and valerian root dry extracts in solid oral dosage forms

İlker DEMİRBOLAT [1, 2] *, Murat KARTAL [1]

[1] Center of Education, Practice and Research in Phytotherapy, Bezmialem Vakıf University, 34093 Fatih, İstanbul, Turkey.

[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, 34668 Haydarpaşa, İstanbul, Turkey.

* Corresponding Author. E-mail: idemirbolat@bezmialem.edu.tr (İ.D.); Tel. +90-216-523 22 88-18 81.

Received: 18 March 2019 / Revised: 10 June 2019 / Accepted: 12 June 2019

ABSTRACT: Minor depression and sleep disorders are common problems in modern societies. There are prescription medicines and herbal remedies to ease the symptoms. St. John's wort is often used to treat mild depression and valerian root is recommended as a sedative for centuries. Nowadays there is an increasing interest for herbal medicines and therefore the herbal products are becoming more and more popular. As dietary supplement retailers selling low quality products -or even don't contain what's claimed on the label- quality becomes the major concern. The legislation to conventional medicines applies to traditional herbal medicines too, which makes registered herbal medicines reliable in production and quality. A CTD application dossier which contains quality data in module 3 should be provided while registering a herbal medicine in EU. In this study we developed a stable formulation and validated two separate methods to quantify the herbal extract amounts in solid oral dosage forms which contains St. John's Wort and valerian dry extract as active ingredients to comply CTD module 3 requirements.

KEYWORDS: St. John's Wort; valerian; validation; formulation; stability.

1. INTRODUCTION

Aerial parts of St. John's Wort (*Hypericum perforatum* L.), a member of genus Hypericum L., has long been used for its health benefits. Hippocrates and Galen suggested this herb for intestinal worms, wound-healing agent and as antimalarial. Paracelsus recommended the herb for mild depression and melancholy [1, 2]. Dry extract of St. John's Wort (SJW) is indicated for symptomatic treatment of mild depressive episodes. Other therapeutic indications for traditional and well-established uses of SJW were mentioned in European Medicines Agency (EMA) 2008 report [3].

Underground parts of *Valeriana officinalis* L. is the drug so called valerian. Hippocrates recommended its medicinal properties for digestive disorders, nausea, and menstrual cramps. Galen was the first who described the sleep-aid properties [4-6]. In German Commission E monographs, the valerian root extract is an approved herbal medicine for its sedative and sleep-aid activity [7]. Valerian root dry extract (VDE) is also used to treat sleep disorders, temporary insomnia, mood disorders and mental stress as described in EMA report [8].

In European Pharmacopoeia (EP), SJW is quantified to antraquinone derivatives called hypericins. EP content range for total hypericins is between 0.1 %and 0.3%, expressed as hypericin [9]. VDE is also described in EP and the content is standardised to minimum 0.25 percent of sesquiterpenic acids, expressed as valerenic acid [10].

Traditional herbal medicinal products are regulated by international or local authorities same way as the conventional medicines. Registration of herbal and conventional medicines requires application dossiers known as common technical document (CTD), a mandatory format for new drug applications. The CTD consists of five modules and the third one contains all the required manufacturing and production quality data about the product [11, 12].

How to cite this article: Demirbolat İ, Kartal M. Formulation, stability and analytical method validations of combined St. John's wort and valerian root dry extracts in solid oral dosage forms. J Res Pharm. 2019; 23(5): 812-821.

ISSN: 2630-6344

https://doi.org/10.35333/jrp.2019.29

There are several traditional herbal medicinal products which contains SJW and VDE together with the aim of working synergistically. In this study we developed a stable oral formulation and validated two separate HPLC methods to quantify hypericins and valerenic acids.

2. RESULTS AND DISCUSSION

2.1 Optimization of HPLC methods for hypericins and valerenic acids

Quantifying hypericins content of SJW according to EP monograph relies on octadecylsilyl (C18) silica gel columns. Various C18 columns were used to achieve the best separation and peak shapes using the same mobile phase as EP suggested. With higher carbon loaded columns peak tailings were unacceptable so a 9.8% carbon loaded 300 mm, 3.9 mm x 10 µm column was selected as the analytical column.

Method described in the EP for VDE was not suitable to quantify valerenic acids in SJW matrix and there is no defined method in the literature either. So a relatively long method was developed to achieve the separation of acetoxyvalerenic acid from the phenolic fraction of SJW with a 15% carbon loaded 250 mm, 4.6 mm x 5 µm C18 column. Both method parameters were given in Table 1.

Table 1. Method parameters for hypericins and valerenic acids methods.

	Hypericins	Valerenic Acids	
System	Shimadzu HPLC Prominence	Shimadzu HPLC Prominence	
Column	Waters µBondapak C18, 300 mm x 3.9 mm x 10 µm	GLSciences ODS-3, 250 mm x 4.6 mm x 5 µm	
Flow	1 mL/min	1.5 mL/min	
Detection	590 nm	220 nm	
Enjection	20 µL	20 µL	
Column Oven	40°C	40°C	
Mobil Phase	Ethyl acetate:Buffer Solution:Methanol (39:41:160 V/V/V).	Mobile Phase A: 5g/L H_3PO_4 Mobile Phase B: Acetonitrile	
Buffer Solution	15.6 g/L NaH_2PO_4 pH: 2 adjusted with H_3PO_4		
Flow Rate	Isocratic	Time (min)	Mobil Phase B (V/V%)
		0 - 1	45 → 45
		1-25	45 → 55
		25 – 40	55
		40 – 41	55 → 45
		41 - 50	45

2.2 Method validation

Method validation was performed according to International Conference on Harmonization (ICH) Q2(R1) Validation of Analytical Procedures: Text and Methodology guidelines [13].

2.2.1. Product and placebo

The products are manufactured according to the formulation given in Table 2.

Table 2. Unit formula per capsule.

Ingredient	Amount for one capsule (mg)
St John's Wort Dry Extract	300
Valerian Root Dry Extract	100
Maldex Pharma (Maltodextrine)	90
Ligamed MF-2-V (Magnesium stearate)	5
HDK N20 Pharma (Silicon dioxide)	5
TOTAL	500

2.2.2. Specificity

The ICH documents define specificity as the ability to assess the analyte in the presence of matrix components. To achieve specificity for both methods; solvents, mobile phases, placebos of the related methods and reference substances (CRS) were used. Specificity results and the chromatograms of the products are represented in Table 3, Table 4 and Figure 1 respectively.

Table 3. The results illustrating specificity of SJW hypericins.

Sample	Pseudohypericin Retention Time	Hypericin retention time	Resolution
SJW dry extract CRS	5.32	10.08	9.08
Placebo	-	-	-
Solvent (Methanol)	-	-	-
Mobile Phase	-	-	-
Product	5.32	10.08	9.1

Table 4. The results illustrating specificity of VDE valerenic acids.

Sample	Acetoxyvalerenic acid retention time	Valerenic acid retention time	Resolution
Valerian dry extract CRS	17.65	35.39	29.7
Placebo	-	-	-
Solvent (Methanol)	-	-	-
Mobile Phase	-	-	-
Product	17.69	35.43	29.7

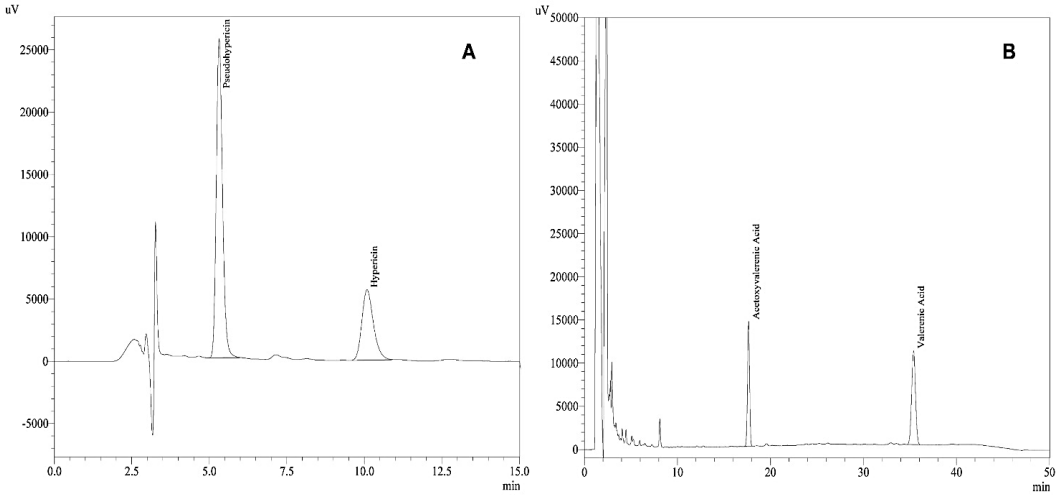

Figure 1. Chromatogram of (A) hypericins of SJW and (B) valerenic acids of VDE standards.

2.2.3. System repeatability, system suitability, range and linearity

To achieve system repeatability, system suitability and linearity; 50%, 80%, 100%, 120% and 150% concentration of SJW and VDE reference substances (CRS) were prepared according to the method described in material and methods section. 100% concentration solution was injected six times to achieve both linearity, system repeatability and system suitability. Table 5 and 6 illustrate the results for both hypericins and valerenic acids while Figure 2 demonstrates the calibration curves for linearity tests. Acceptance for the range test was based on the following: range of the method should be linear, accurate, and repeatable between the 80% and the 120% of the solution and the results comply with the criteria.

Table 5. System repeatability, system suitability, range and linearity data for hypericins of SJW between concentration and peak areas. The calculated RSD% was ≤ 2.0 for system repeatability and the system suitability tests comply with the criteria.

Concentration	Pseudohypericin area	Hypericin area	Total hypericins area	Average hypericins area
50% (35 mg SJW dry extract CRS)	151099	61191	212290	211998
	150681	61006	211687	
	150751	61267	212018	
80% (56 mg SJW dry extract CRS)	243532	99401	342933	343160
	243669	99415	343084	
	243673	99791	343464	
100% (70 mg SJW dry extract CRS)	304697	125046	429743	430014
	304941	124843	429784	
	305332	124904	430236	
	305073	124913	429986	
	305147	125309	430456	
	305064	124813	429877	
120% (84 mg SJW dry extract CRS)	368094	151735	519829	519182
	368249	150212	518461	
	368291	150965	519256	
150% (105 mg SJW dry extract CRS)	460169	189256	649425	649339
	460267	189190	649457	
	459870	189264	649134	

Table 6. System repeatability, system suitability, range and linearity data for valerenic acids of VDE between concentration and peak areas. The calculated RSD% was ≤ 2.0 for system repeatability and the system suitability tests comply with the criteria.

Concentration	Axetoxyvalerenic acid area	Valerenic acid area	Total valerenic acids area	Average valerenic acids area
50% (25 mg Valerian dry extract CRS)	113532	157834	271366	272100
	114394	158613	273007	
	113668	158258	271926	
80% (40 mg Valerian dry extract CRS)	184978	256604	441582	441660
	184658	256646	441304	
	185204	256889	442093	
100% (50 mg Valerian dry extract CRS)	224913	313390	538303	538765
	224953	312923	537876	
	225483	313181	538664	
	225093	312730	537823	
	226156	313833	539989	
	225971	313962	539933	
120% (60 mg Valerian dry extract CRS)	269122	373887	643009	643572
	269334	374059	643393	
	269358	374957	644315	
150% (75 mg Valerian dry extract CRS)	334234	464471	798705	801567
	335851	467361	803212	
	335852	466933	802785	

2.2.4. Accuracy

The accuracy of method was determined by means of recovery data. For this purpose units were produced according to Table 13 in materials and methods section. Three sets of samples were prepared for each concentration and each sample tested for twice. The amount of extracts per capsules was calculated according to the formulas given below. Table 7 and 8 demonstrate the accuracy studies.

$$SJW\ in\ Capsules\ (g) = \frac{(A1 + A2) \times m2 \times p2}{A3 \times m1 \times p1} \quad \text{(Eq. 1)}$$

A1: area of pseudohypericin in product, A2: area of hypericin in product, m1: sample in test solution in grams, p1: percentage of total hypericins in raw material, A3: area of hypericin in the CRS, m2: CRS in in reference solution in grams, p2: percentage of hypericin in CRS. (Total hypericins in the raw materials should be quantified before use).

$$VDE\ in\ Capsules\ (g) = \frac{(A1 + A2) \times m2 \times p2}{A3 \times m1 \times p1} \quad \text{(Eq. 2)}$$

A1: area of acetoxyvalerenic acid in product, A2: area of valerenic acid in product, m1: sample in test solution in grams, p1: percentage of total valerenic acids in raw material, A3: area of valerenic acid in the CRS, m2: CRS in reference solution grams, p2: percentage of valerenic acid in CRS. (Acetoxyvalerenic acid and valerenic acids in the raw materials should be quantified as valerenic acid before use).

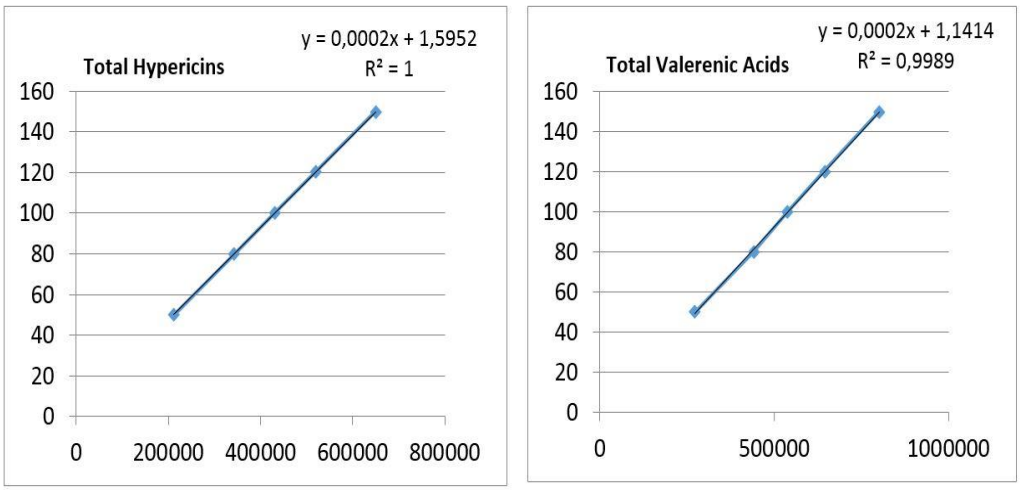

Figure 2. Calibration curve for hypericins and valerenic acids.

Table 7. Accuracy studies for SJW.

Sample Concentration	SJW in Capsules (mg)	Analysed SJW in Capsules (mg)	Recovery %
80%	240	242.90	101.2
	240	241.58	100.7
80%	240	244.22	101.8
	240	242.90	101.2
80%	240	245.28	102.2
	240	245.01	102.1
100%	300	299.74	99.9
	300	299.74	99.9
100%	300	300.53	100.2
	300	299.48	99.8
100%	300	302.11	100.7
	300	301.58	100.5
120%	360	362.37	100.7
	360	362.10	100.6
120%	360	358.95	99.7
	360	359.74	99.9
120%	360	364.47	101.2
	360	363.68	101.0

Table 8. Accuracy studies for VDE.

Sample Concentration	VDE in Products % (w/w)	Analysed VDE in Products % (w/w)	Recovery %
80%	80	80.78	100.9
	80	81.44	101.8
80%	80	79.78	99.7
	80	79.89	99.9
80%	80	80.33	100.4
	80	81.22	101.5
100%	100	101.89	101.9
	100	101.56	101.6
100%	100	99.11	99.1
	100	99.56	99.6
100%	100	101.44	101.4
	100	101.22	101.2
120%	120	120.11	100.1
	120	119.89	99.9
120%	120	120.33	100.3
	120	120.22	100.2
120%	120	120.56	100.5
	120	120.78	100.6

2.2.5. Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. Precision of the method assessed in terms of repeatability, intermediate precision and reproducibility. Reproducibility refers to the use of the analytical procedure in different laboratories, as in a collaborative study. Intermediate precision (also known as ruggedness) expresses within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment. Table 9 shows intermediate precision and repeatability data while Table 10 demonstrates the reproducibility of the method.

Table 9. The assessment of repeatability and intermediate precision for SJW and VDE.

SJW in Capsules (mg)	Analyst-1	Analyst-2
300	304	305
300	303	302
300	295	298
300	298	302
300	301	303
300	307	306
Statistics		
Mean±SD	300±4.32	300±2.66
RSD%	1.43	0.93
VDE in Capsules (mg)	**Analyst-1**	**Analyst-2**
100	102	98
100	102	99
100	98	102
100	101	100
100	99	101
100	98	97
Statistics		
Mean±SD	100±1.89	100±1.87
RSD%	1.89	1.88

Table 10. Data obtained from reproducibility study of SJW and VDE using same type columns with different serial numbers.

SJW in Capsules (mg)	Analyst-1
300	301
300	303
300	298
300	301
300	305
300	302
Statistics	
Mean±SD	300±2.33
RSD%	0.77
VDE in Capsules (mg)	**Analyst-1**
100	100
100	98
100	103
100	102
100	99
100	102
Statistics	
Mean±SD	100±1.96
RSD%	1.95

2.2.6. Limit of Detection and Limit Quantification (LOD and LOQ)

Analytical procedures for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products are classified as category 1. United States Pharmacopoeia states that LOD and LOQ test data elements are not required for this category [14].

2.2.7. Robustness

The robustness of an analytical procedure is the measure of method capacity to remain stable with relatively small amount of changes in method parameters [14]. Robustness test were evaluated by increasing the flow rate at 10% and increasing the temperature of the column for 5°C. Table 11 shows the data of robustness tests including difference of the results as percentage.

Table 11. The results illustrating robustness test data for hypericins and valerenic acids.

Method	Normal Method	Flow Increased Method	Difference (%)
Hypericins Method	300 mg	302 mg	0.66
Valerenic Acids Method	100 mg	101 mg	1.00
Method	**Normal Method**	**Temperature Increased Method**	**Difference (%)**
Hypericins Method	300 mg	297 mg	1.00
Valerenic Acids Method	100 mg	101 mg	1.00

2.2.8. Solution stability

Test solutions used in accuracy tests were kept in 4^0C and 25^0C in the dark for 48 hours and analysed again to achieve solution stability. Both hypericins and valerenic acids remained stable in the solution.

2.3. Formulation stability

Accelerated and long term stability tests were carried out to confirm the stability of the herbal product in primary packaging material according to ICH Q1A(R2); Stability testing of new drug substances and products guidelines [15]. Accelerated stability conditions were as follows; 40 ± 2°C, 75% ± 5% R.H meanwhile long term stability conditions were 25 ± 2°C, 60% ± 5% R.H. Table 12 shows the accelerated stability test results. Long term stability control test periods were 1, 3, 6, 9, 12, 18, 24th months and the results were in the limits.

Table 12. Accelerated stability data.

Product Specifications		Time Periods		
Tests	Limits	1st month	3rd month	6th month
Average Capsule Weight	500 ± 7.5%	498.27	501.36	500.84
Uniformity of Weight	500 mg ± 7.5% (not more than 2 capsules deviate from the average mass) 500 ± 15% (none of the tablets capsules from the average mass)	Confirms	Confirms	Confirms
Disintegration	Maximum 15 minutes	Confirms	Confirms	Confirms
Moisture (Karl Fischer)	Maximum 3%	2.13%	2.21%	2.58%
Assay (SJW)	300 ± 30 mg	301 mg	298 mg	302 mg
Assay (VDE)	100 ± 10 mg	98 mg	97 mg	99 mg

2.4. Physical properties

Disintegration tests of capsules were performed with a PTZ-S single basket tablet disintegrator (Pharma Test Apparatebau AG/Germany). All the capsules were disintegrated less than 15 minutes. A dissolution test was not reported in this study because disintegration is shown to be more discriminating than dissolution as stated by European Medicine Agency [16].

3. CONCLUSION

SJW also known as the herbal prozac is gaining popularity among with the herbal medicines. Valerian, on the other hand, has been used for its sedative properties for centuries. There are a lot of dietary supplements containing both SJW and VDE in the market but their quality is always questionable by comparison with the traditional herbal medicinal products. The following study demonstrates a stable oral formulation and a fully validated analytical method to comply the related parts of CTD could be used registering combined SJW and VDE in oral dosage form as traditional herbal medicinal product.

4. MATERIALS AND METHODS

Methanol, ethyl acetate, sodium dihydrogen phosphate, orthophosphoric acid and acetonitrile were purchased from Merck (Darmstadt, Germany). St. John's wort dry extract CRS and valerian root dry extract CRS were purchased from EDQM (Strasburg, France). EP grade St. John's wort quantified dry extract (0.19% total hypericins by weight) and valerian root dry extract (0.45 total valerenic acids by weight) were purchased from Naturex (Avignon, France). All Naturex extracts were quantified with EDQM reference substances before using as raw materials. The methods were developed and validated using a Shimadzu HPLC Prominence (Kyoto, Japan) system coupled with a PDA detector. Columns were purchased from GLSciences (Japan) and Waters (USA). Parameters for both methods are listed in Table 1. Hitachi Aquacounter AQV 300 (Tokyo, Japan) was used to quantify water amount in the formulations during stability studies.

4.1. Formulating and preparation of the dosage forms

Unit formula of the dosage form is given in Table 2. Herbal extracts and the rest of the excipients were mixed for 20 minutes in a V-type mixer and size 0 capsules were filled with 500 mg of bulk product using a Karnavati Minicap (Gujarat, India) capsule filling machine. Amber glass containers with plastic caps and silica gel (1.75 gram) desiccant were used as primary packaging material for 60 capsules.

Placebo for SJW was prepared by increasing the amount of maltodextrin to 390 mg per capsule while placebo for valerian was prepared with 190 mg maltodextrin. Placebos were used for specificity tests in method validations. Accuracy test samples were prepared according to Table 13.

Table 13. Accuracy study sample formulations per capsule.

Ingredient	80% SJW	120% SJW	80% VDE	120% VDE
SJW Dry Extract (mg)	240	360	300	300
Valerian Root Dry Extract (mg)	100	100	80	120
Maltodextrine (mg)	150	30	110	70
Magnesium stearate (mg)	5	5	5	5
Silicon dioxide (mg)	5	5	5	5
TOTAL	500	500	500	500

4.2. Assay method for hypericins

Preparing the test solutions requires 225 mg of the capsule content introduced into a 50 mL volumetric flasks and filled with 40 mL of methanol. After sonicating for 10 minutes and cooling to the room temperature flasks were filled up to the volume with methanol. Test solutions were filtered through a 50 µm PTFE membrane filter into analytical vials and the vials were subjected to 765 W/m2 Xenon lamp light for 10 minutes converting protohypericins to hypericins. After cooling to the room temperature the 20 µL of the solutions were injected to HPLC system according to the method parameters described in Table 1.

Reference solutions were prepared same way except 70 mg of SJW dry extract CRS was added in 25 mL volumetric flask.

To achieve the system suitability, resolution between pseudohypericin and hypericin should be 8-9 and the RSD% of total hypericins areas sould be ≤2 for two sequential reference solution injection. The amount of SJW could be calculated with the formula given in *2.2.4 Accuracy* section.

4.3. Assay method for valerenic acids

To prepare the test solutions for valerenic acids, one capsule content was introduced into a 50 mL volumetric flask. Flask were filled with 40 mL of methanol and sonicated for 10 minutes. After cooling to the room temperature the flasks were filled up to the volume with methanol. Test solution were filtered through a 50 µm PTFE membrane filter into an analytical vial and 20 µL of the solutions were injected to HPLC system according to the method parameter given in Table 1..

Reference solutions were prepared same way except 50 mg of valerian dry extract CRS was added in 25 mL volumetric flask.

To achieve the system suitability, RSD% of total valerenic acid (valerenic acid and acetoxyvalerenic acid) areas should be ≤2 for two sequential reference solution injection. The amount of VDE in dosage forms could be calculated with the formula given in *2.2.4 Accuracy* section. Figure 3. represents the chromatograms obtained from the final products.

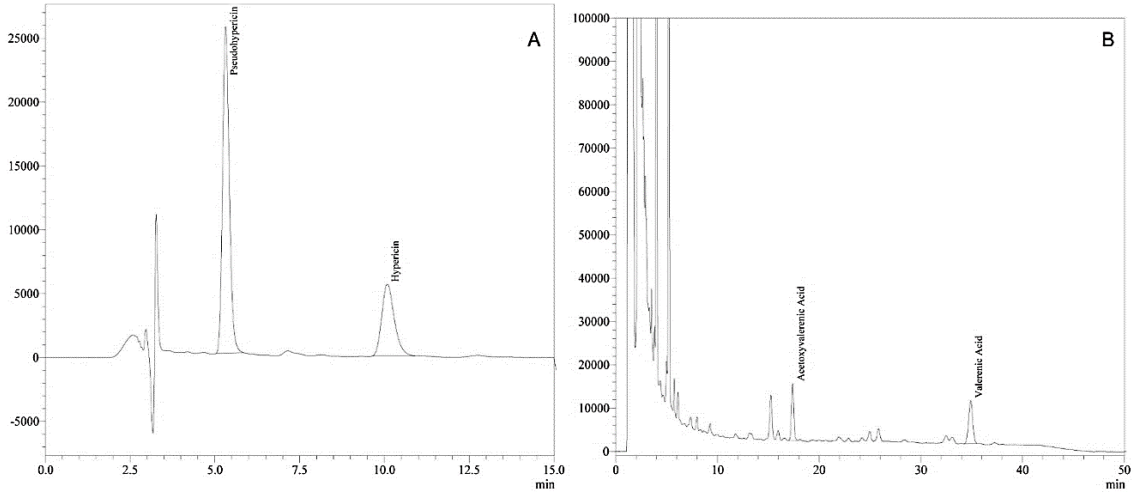

Figure 3. Chromatogram of (A) hypericins of SJW and (B) valerenic acids of VDE in final product.

4.4. Stability studies

Accelerated and long term stability tests for products in primary packaging material were performed in a Binder KBF 115 (Tuttlingen, Germany) stability chamber. The conditions and sampling periods were described in 2.3 *Formulation Stability* section.

Author contributions: Concept – İ.D., M.K.; Design – İ.D., M.K.; Supervision – M.K.; Materials – İ.D., M.K.; Data Collection and/or Processing – İ.D., M.K.; Analysis and/or Interpretation – İ.D., M.K.; Literature Search – İ.D., M.K.; Writing – İ.D., M.K.; Critical Reviews – İ.D., M.K.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Redvers A, Laugharne R, Kanagaratnam G, Srinivasan G. How many patients self-medicate with St John's wort? Psychiatr Bull. 2001; 25(7): 254–256 [CrossRef]

[2] Clement K, Covertson C, Johnson M. J, Dearing K. St. John's wort and the treatment of mild to moderate depression: A systematic review. Holist Nurs Pract. 2006; 20(4):197–203.

[3] Community Herbal Monograph on Hypericum perforatum L. Herba. EMA/HMPC/101304/2008. European Medicines Agency, London, 2008.

[4] Grieve M. A Modern Herbal. Vol 2. New York, NY: Hafner Press; 1974: 824-830.

[5] Blumenthal M, Goldberg A, Brinckmann J. Herbal Medicine Expanded Commission E Monographs. Newton, MA: Integrative Medicine Communications; 2000: 394-400.

[6] Turner W. A New Herbal, Parts II and III. Cambridge, England: Cambridge University Press; 1995: 464-466.

[7] Ross SM. Psychophytomedicine: An Overview of Clinical Efficacy and Phytopharmacology for Treatment of Depression, Anxiety and Insomnia. Holist Nurs Pract. 2014; 28(4): 275-280. [CrossRef]

[8] European Union herbal monograph on Valeriana officinalis L., radix. EMA/HMPC/150848/2015. European Medicines Agency, London, UK, 2016.

[9] Council of Europe. St. John's Wort Quantified Dry Extract (01/2013:1874). European Pharmacopoeia 8th edition. Strasbourg, France, 2013.

[10] Council of Europe. Valerian Dry Hydroalcoholic Extract (01/2011:1898). European Pharmacopoeia 8th edition. Strasbourg, France, 2013.

[11] International Conference on Harmonization (ICH), ICH M4; Organisation of the common technical document for the registration of pharmaceuticals for human use, Geneva, Switzerland, 2016.

[12] International Conference on Harmonization (ICH), ICH M4Q(R1); Organisation of the common technical document for the registration of pharmaceuticals for human use, Geneva, Switzerland, 2002.

[13] International Conference on Harmonization (ICH), ICH Q2(R1); Validation of Analytical Procedures: Text and methodology, Geneva, Switzerland, 2005.

[14] General Information / (1225) Validation of Compendial Procedures. United States Pharmacopeia and National Formulary (USP 41-NF 36). Rockville, MD: United States Pharmacopeial Convention; 2016.

[15] International Conference on Harmonization (ICH), ICH Q1A(R2); Stability Testing of New Drug Substances and Products, Geneva, Switzerland, 2003.

[16] Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products /traditional herbal medicinal products. EMA/CPMP/QWP/2820/00 Rev. 2. European Medicines Agency, London, UK, 2011.