Optimization of Extraction Conditions for Active Components in *Hypericum perforatum* Using Response Surface Methodology

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Optimal conditions for extraction of *Hypericum perforatum* were determined using response surface methodology. A 3 × 4 × 4 full factorial design representing three extraction temperatures, four extraction times, and four solvent concentrations was executed. The overall extraction efficiency was defined by comparing either the total extractable material weight or the individual component peak area to the peak area of luteolin as internal standard. Of the tested variables, the extraction temperature most significantly affected extraction efficiency. Higher temperatures gave better extraction efficiencies, but high temperature also caused decomposition of hypericin. Within the test range, responses for most variables had local maxima. Optimum ranges of time and concentration for individual variables were overlaid. Considering all variables, optimum ranges for extraction time and extraction solvent concentration (percent ethanol in acetone) were 5.0−6.7 h and 44−74% at 23 °C, 5.4−6.9 h and 45−72% at 40 °C, and 5.3−5.9 h and 44−69% ethanol in acetone at 55 °C, respectively.

Keywords: *Hypericum perforatum; extraction; response surface methodology; optimization*

INTRODUCTION

In recent years, the use of herbs as dietary supplements and over-the-counter drugs has increased dramatically in many countries. The World Health Organization estimates that up to 80% of the world's population relies on traditional medicinal (not Western) systems, and in many of these, herbal medicines play a key role. In the U.S., nearly 60 million people use herbal products (Lindenmaier et al., 1999). Among these products, *Hypericum perforatum* (St. John's Wort) is a very popular one. It has been one of the top five herb remedies in recent years. *H. perforatum*, a perennial herb, is native to Europe, West Asia, and North Africa. It has been naturalized in Asia, Africa, Australia, and North America. In the U.S., it is considered to be a roadside weed (Snow, 1996). *H. perforatum* has been used as a medicinal herb throughout history. It has been reported as an antidepressive, an antiviral, an antimicrobial, an antiinflammatory, and a healing agent (Brolis et al., 1998). *H. perforatum* is one herb that has a proven efficacy/activity as a selective serotonin release inhibitor (SSRI), and in that context it is clearly comparable to orthodox antidepressants (Linde et al., 1996).

H. perforatum contains components belonging to a number of natural product groups which include naphthodianthrones, acylphloroglucinols, flavonol glycosides, biflavones, proanthocyanidins, and phenylpropanes (Erdelmeier, 1998). For analysis of an extract of this complex mixture, the best separation technique is high-performance liquid chromatography (HPLC). Several HPLC methods have been developed focusing on either naphthodianthrones such as hypericin and pseudohypericin which were considered as the major antidepressive ingredients in *H. perforatum* (Helman et al., 1998; Kurth and Spreemann, 1998; Liebes et al., 1991; Micali et al., 1996; Piperopoulos et al., 1997) or the whole extraction mixtures (Brolis et al., 1998; Butterweck et al., 1997; Erdelmeier, 1998; Kurth and Spreemann, 1998; Nahrstedt and Butterweck, 1997). Limited information is available regarding extraction efficiency for this whole herb matrix. Only a few solvents such as methanol and acetone were used. Extraction efficiency is affected by multiple parameters, including temperature, time, and solvent polarity. The effects of these parameters may be either independent or interactive. Defining extraction efficiency becomes more complicated if the target is a complex system, e.g., *H. perforatum* leaves with regard to known, identifiable active components. Active constituents are thought to be hypericin, hyperforin, and even the flavonoids such as rutin. Variations in extraction conditions favor different components in the mixture. Until now, no systematic studies have been published for the optimal extraction of active components. None have investigated the influence of extraction variables on the recovery of active components.

Response surface methodology (RSM) is a powerful experimental procedure for optimizing multiple, interrelated parameters. In this method, experiments are conducted to discover which values of the parameters (referred to as independent variables) optimize a response (dependent variable). The values of dependent variables are measured with each independent variable

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at three or more values. Then a quadratic response surface can be estimated by least-squares regression. The predicted optimal value for the independent variable can be found from the estimated surface, if the surface is shaped like a simple hill (has a maximum stationary point) or a valley (has a minimum stationary point). However, if the estimated surface is more complicated (has a saddle point), or if the predicted optimum is far from the region of experiment, the shape of the surface can be analyzed to indicate the directions in which new experiments should be performed. An example of the specific software for the response surface regress analysis is the RSREG procedure released by the SAS Institute (SAS Institute Inc., 1989). RSM has been successfully used in various multiparameter systems (Bates et al., 1998; Costa et al., 1998; Gong and Chen, 1998; Kapat et al., 1998; Kimmel et al., 1998; Schaffner et al., 1998). The objective of the present work is to achieve an optimal extraction condition for *H. perforatum* leaves by using RSM.

MATERIALS AND METHODS

Materials. Dry St. John Wort leaves (cut and shifted) were purchased from a local natural food store in Little Rock, AR. The package label indicated it was supplied by the Frontier Natural Products Co. No other information was available. The leaves were ground using a coffee blender (Braun, type 4041, Braun Inc., Woburn, MA), passed through a 20 mesh sieve, sealed into a plastic tube, and stored at −60 °C. For protection from light degradation, the complete procedure was performed under yellow light and the plastic tube was covered with aluminum foil.

Reference standards of hypericin, luteolin, rutin, quercetin, and quercitrin were purchased from Sigma Chemical Co. (St. Louis, MO), and hyperoside and isoquercitrin from Indofine Chemical Co., Inc. (Somerville, NJ). Other standards, such as hyperforin and adhyperforin, were not available. All other reagents used were purchased from J. T. Baker (Phillipsburg, NJ) and were of HPLC grade. Ethanol was absolute. Water was distilled, deionized (>18 MΩ/cm), and passed through a carbon filter (Milli-Q water purification system, Waters, Milford, MA).

Experimental Design and Statistical Analysis. The entire experiment was executed in three phases. The objective of phase I was to determine the optimum solvent and then to determine the optimum extraction time of the optimum solvent. In this phase, solvents with a wide range of polarity were tested, which included water, 50% water−ethanol, ethanol, 50% ethanol−acetone, acetone, chloroform, and hexane. The 50% ethanol−acetone solvent combination was also tested at a wide range of extraction times, from 15 min to 24 h. The reason for the selection of this solvent combination (50% ethanol−acetone) is described in the Results and Discussion. Extraction efficiency was estimated by comparing the total extractable material weight and the peak area of individual components measured relative to the area of the internal standard (luteolin) on the sample weight basis (referred to as the peak area ratio). The objective of the phase II study was to obtain the optimum extraction condition (extraction temperature, extraction time, and solvent composition) by using RSM. On the basis of the result of phase I, the phase II experiment was conducted to evaluate a narrower range of extraction times (4.5, 5.5, 6.5, and 7.5 h) and solvent combinations (20%, 40%, 60%, and 80% ethanol in acetone) at three extraction temperatures (23, 40, and 55 °C). Experimental extraction efficiency values were fitted to a response surface model to obtain a global optimum extraction condition based on "critical values". The specific software for the response surface regression analysis is the RSREG procedure of the SAS Proprietary Software Release 6.12 (SAS Institute Inc., 1989). As described in the manual, the RSREG procedure "fits the parameters of a complete quadratic response surface and analyzes the fitted surface to determine the factor levels of optimum response". The critical values are the values of the independent variables (extraction temperature, extraction time, and solvent composition or concentration) that correspond to the stationary point of the fitted response surface. The critical value may occur at a minimum, maximum, or saddle point. The nature of the stationary point indicates the characteristic of the response surface. If the response surface has a maximum stationary point, then at this point the response variable (peak area ratio or extractable material weight) reaches the maximum. The values of the independent variables (extraction temperature, extraction time, and solvent concentration) at this point are referred to as critical values, and these values are the optimum extraction condition. In this study, the response surface model with a maximum stationary point was considered. In phase III, to estimate the accuracy of the response surface models obtained in phase II, an extraction time and solvent combination at each temperature within a 95% confidence range of the critical value were selected to do the extraction. The extraction efficiencies obtained from the experiment were compared with those predicted from the response surface model at each temperature (SAS Institute Inc., 1989).

Table 1. Gradient Profile Used in HPLC for Analysis of *H. perforatum* Extracts

program time (min)	mobile phase A (%) [0.5% trifluoroacetic acid (TCA) in water]	mobile phase B (%) [methanol−acetonitrile−TCA (60:39.5:0.5)]
0	90	10
15	78	22
44	62	38
49	0	100
54	0	100
63	36	64
69	90	10
86	90	10

Sample Preparation. A 2.00 g aliquot of dry *H. perforatum* leaf powder was extracted with 50 mL of solvent in a flask tightly covered with aluminum foil for the designated time in an orbital water bath shaker (model 3540, Lab-Line Instruments, Inc., Melrose Park, IL) at the designated temperature. After being taken out of the water bath shaker, the flask stood at room temperature for 10 min before the extraction was filtered through no. 4 filter paper (Whatman International Ltd., Maidstone, England). The residues were extracted twice more with 20 mL of the same solvent under the same conditions. The filtrates of the three extractions were combined together in a 100 mL volumetric flask, and solvent was added to make the final volume. A 3.0 mL portion of this solution was pipetted into a preweighed test tube (13 × 100 mm). The solvent was evaporated in a vacuum centrifuge concentrator (SpeedVacPlus, SC 110A, Savant Instruments, Inc., Holbrook, NY) for 4 h, and then the remaining solvent was evaporated under a stream of nitrogen for 1.5 h. After weighing, the residue was dissolved in methanol to give a final concentration of 100 mg of dry weight/mL. Eighty microliters of methanol, 20 μL of luteolin (1.6 mg/mL; used as an internal standard), and 100 μL of the sample methanol solution were mixed together and filtered through a 0.45 μm Nylon syringe filter (Osmonics, Minnetonka, MN) for HPLC analysis. The UV−vis spectrum of luteolin (internal standard) is similar to those of other flavonoids in the *H. perforatum* extract; however, it was completely separated from other components in the HPLC procedure.

HPLC Analysis. A Waters 600 liquid chromatograph (Waters Associates, Milford, MA) with a photodiode array (PDA) detector and 714 autoinjector was used. The instrument control and data processing were accomplished with Millennium 2010 Chromatogram Manager software (Waters Chromatography Division, Millipore Corp., Milford, MA). Twenty microliters of HPLC sample solution containing the internal standard was injected onto a S5 ODS2 column (25 cm × 4.6 mm, 5 μm, Phase Separations, Norwalk, CT). Detection was

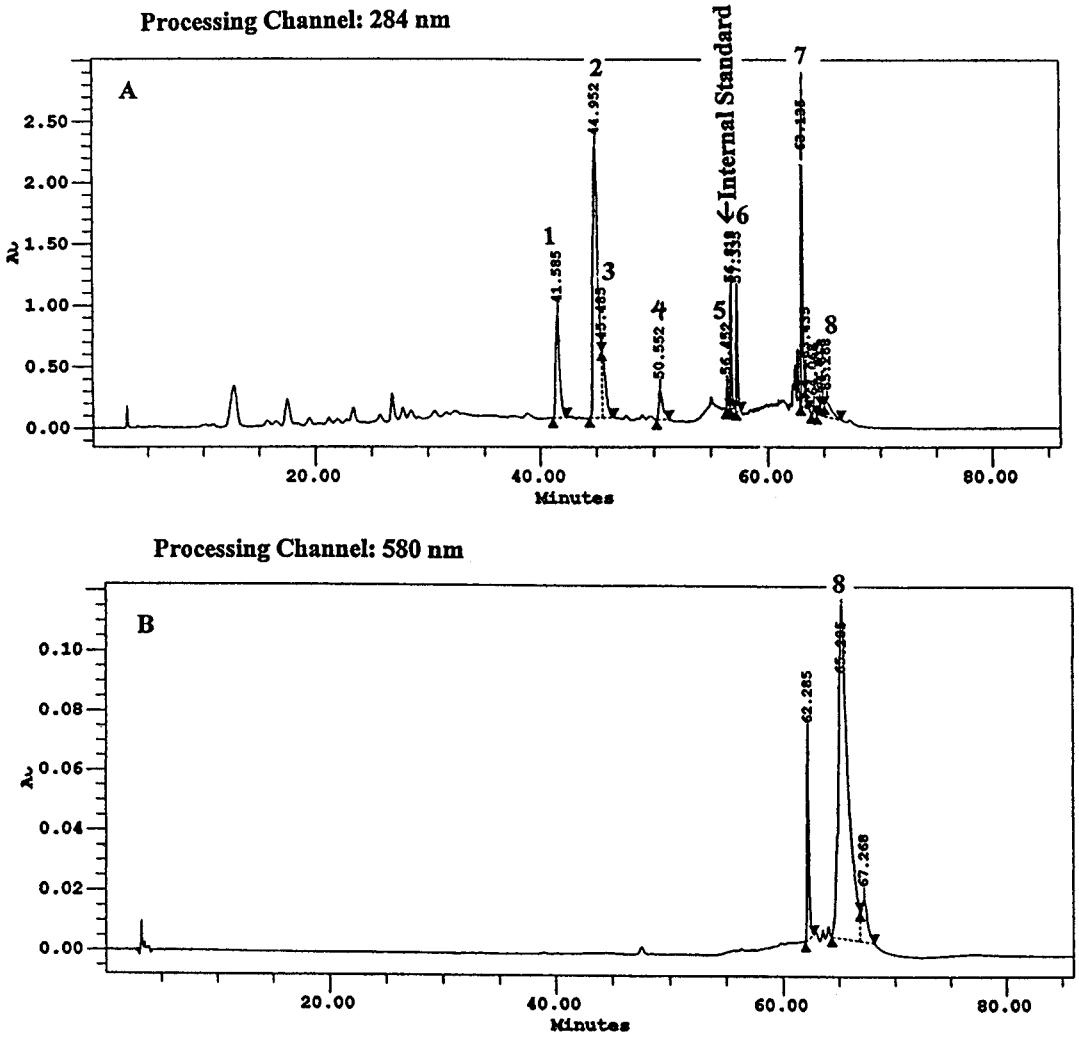

Figure 1. Typical HPLC chromatograms of the *H. perforatum* extraction: solvent, 60% ethanol in acetone; extraction time, 5.7 h; extraction temperature, 23 °C; (A) processing channel, 284.0 nm; (B) processing channel, 580.0 nm; (1) unknown; (2) rutin; (3) isoquercitrin; (4) quercitrin; (5) quercetin; (6) unknown; (7) unknown; (8) hypericin.

Table 2. Influence of Different Solvents on the Extraction Efficiency

		HPLC peak area ratio per gram of sample weight[b]							
solvent	EMW[a] (g/g)	P1	P2	P3	P4	P5	P6	P7	P8
water	0.192	0.056	ND	ND	ND	ND	ND	ND	ND
water−ethanol (50%)	0.53	0.29	0.32	0.30	0.12	0.60	0.16	0.34	0.01
ethanol	0.25	0.32	0.72	0.92	0.16	0.05	0.20	1.06	0.04
ethanol−acetone (50%)	0.16	0.30	0.66	0.90	0.16	0.02	0.20	1.07	0.07
acetone	0.15	0.36	0.88	0.66	0.21	0.02	0.20	0.98	0.07
chloroform	0.03	ND	ND	ND	ND	ND	ND	1.14	ND
hexane	0.02	ND	ND	ND	ND	ND	ND	1.59	ND

[a] EWM = extractable material weight, g/g. [b] Peak area of the sample component as the relative response to the internal standard: (P1, P6, and P7) unknown; (P2) rutin; (P3) isoquercitrin; (P4) quercitrin; (P5) quercetin; (P8) hypericin.

at 284 nm for most components and 580 nm for naphthodianthrones. The mobile phase consisted of two components, (A) 0.5% trifluoroacetic acid in water and (B) methanol−acetonitrile−trifluoroacetic acid (60:39.5:0.5), and followed the gradient program in Table 1. The total mobile phase flow rate was 1 mL/min.

For qualitative purposes, retention times and UV−visible spectrums of standards and sample peaks were regularly monitored. The purity of each sample peak was also checked by PDA software (Waters Chromatography Division, Millipore Corp.). For quantitative analysis, the peak area of each selected component was measured by using the Millennium software (Waters Chromatography Division, Millipore Corp.). The HPLC detector response ratios of the individual components to the internal standard (peak area ratios) were further calculated on the basis of the sample weight. These corrected peak area ratios were used in statistical analysis.

RESULTS AND DISCUSSION

Phase I. Selection of Solvents and Extraction Times. A typical HPLC chromatogram of the *H. perforatum* extraction is presented in Figure 1. By comparison of the retention time and PDA spectrums with the reference standards, five peaks were identified. They were (P2) rutin, (P3) isoquercitrin, (P4) quercitrin, (P5) quercetin, and (P8) hypericin. It was difficult to detect the hypericin peak when 284 nm was chosen as the

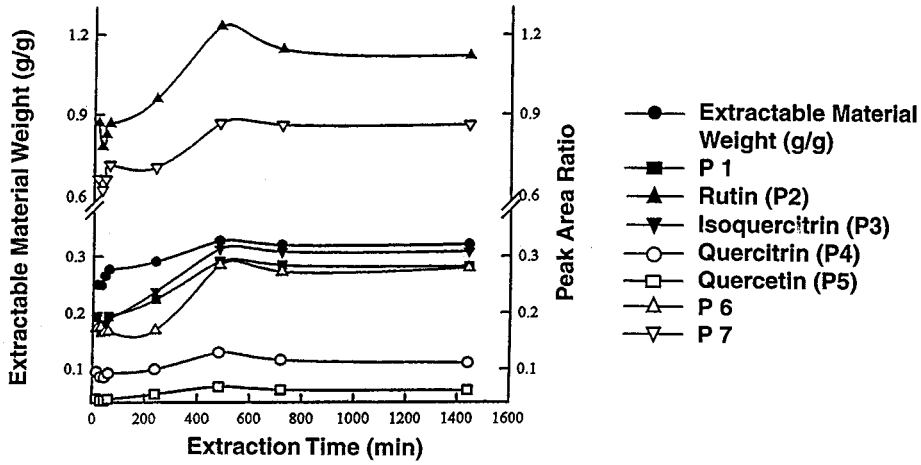

Figure 2. Influence of extraction time on the extraction efficiency, expressed as extractable material weight and peak area ratios of selected compounds: peak 1 (P1), rutin (P2), isoquercitrin (P3), quercitrin (P4), quercetin (P5), peak 6 (P6), and peak 7 (P7).

Table 3. Critical Values of Time, Concentration, and Temperature Based on the Response Surface Model and Situation of Each Response Variable at Each Stationary Point

			HPLC peak area ratio per gram of sample weight [b]							
		EMW[a] (g/g)	P1	P2	P3	P4	P5	P6	P7	P8
			Time, Concentration, and Temperature							
critical value	time (h)	5.8	6.0	6.0	6.0	6.2	6.5	0.2	5.2	1.9
	concn (%)	64	64	61	62	54	44	115	65	47
	temp (°C)	11.0	25.6	26.4	24.5	18.4	−6.4	268	65.5	−0.8
stationary point	quality[c]	S	S	S	S	S	S	max	max	S
	$value_{pred}$[d]	0.236	1.14	4.16	0.82	0.36	0.08	1.51	2.03	0.35
			Time and Concentration at 23 °C							
critical value	time (h)	5.6	6.0	6.0	5.5	6.0	5.3	5.4	5.3	3.2
	concn (%)	59	63	63	61	63	57	61	62	64
stationary point	quality[c]	max	max	max	max	max	max	max	max	S
	$value_{pred}$[d]	0.248	1.06	3.74	0.78	0.32	0.09	0.40	1.42	0.31
			Time and Concentration at 40 °C							
critical value	time (h)	6.2	6.1	5.9	10.2	5.4	3.9	5.6	8.5	6.0
	concn (%)	60	90	69	151	58	54	56	144	56
stationary point	quality[c]	max	max	max	max	max	S	max	max	S
	$value_{pred}$[d]	0.256	1.14	4.03	0.93	0.37	0.11	0.56	1.88	0.25
			Time and Concentration at 55 °C							
critical value	time (h)	5.9	5.7	5.7	5.6	5.6	5.7	5.7	5.6	5.4
	concn (%)	59	71	63	70	63	63	65	59	53
stationary point	quality[c]	max	max	max	max	max	max	max	max	max
	$value_{pred}$[d]	0.325	1.92	6.82	1.30	0.57	0.24	0.86	2.28	0.36

[a] EWM = extractable material weight, g/g. [b] Peak area of the sample component as the relative response to the internal standard: (P1, P6, and P7) unknown; (P2) rutin; (P3) isoquercitrin; (P4) quercitrin; (P5) quercetin; (P8) hypericin. [c] Quality: S, saddle; max, maximum. [d] $Value_{pred}$ = value calculated from the response surface model.

detection wavelength because other compounds (such as rutin and quercitrin) in the extract were much more concentrated than hypericin, and these compounds had greater molar absorbency at this wavelength. When 580 nm was chosen as the detection wavelength, the other compounds had little absorbency, and hypericin had its maximum. Therefore, at 580 nm, the hypericin peak became the largest peak in the chromatogram. P1, P6, and P7 were not identified at this time because of the lack of suitable standards.

The effect of various solvents on the extraction efficiency is shown in Table 2. In general, solvents with very high solvent polarity, such as water, or very low solvent strength, such as chloroform and hexane, did not give good extraction results. With water extraction only P1 was detectable. Although the solvent combination of 50% ethanol−water gave the highest extractable material weight, it was not efficient for extraction of the components that were analyzed. In low polarity solvent (chloroform and hexane) extractions, P1, rutin, isoquercitrin, quercitrin, quercetin, P6, and hypericin were not detectable; however, hexane achieved the greatest concentration of P7. Solvents with moderate solvent polarity had more universal extraction capabilities. All selected peaks were detectable in water−ethanol, ethanol, ethanol−acetone, and acetone extractions. For most peaks, these solvents gave higher extraction efficiency when compared with other solvents (Table 2). Most components in *H. perforatum*, such as naphthodianthrones, flavonol glycosides, and biflavones, are more soluble in moderately polar solvents. Some components such as phloroglucinols including hyperforin and adhyperforin are more lipophilic, and although they dissolve in moderately polar solvents, they have better solubilities in weak polar or nonpolar solvents. Peak 7 apparently followed this pattern. In consideration of the general situation, the moderately polar solvent (50% ethanol−acetone combination) was chosen for the phase

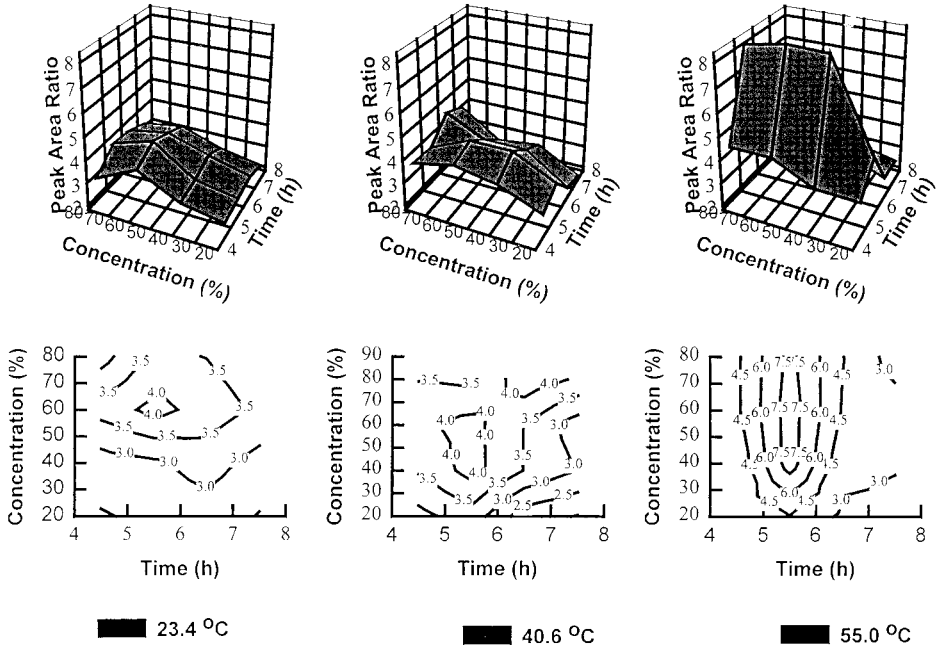

Figure 3. Influence of extraction time and solvent concentration (% ethanol in acetone) on the peak area ratio of rutin at different extraction temperatures. The three-dimensional graphs on the top part of the figure were plotted by using the experimental data. The contour plots on the bottom part of figure were based on the response surface models.

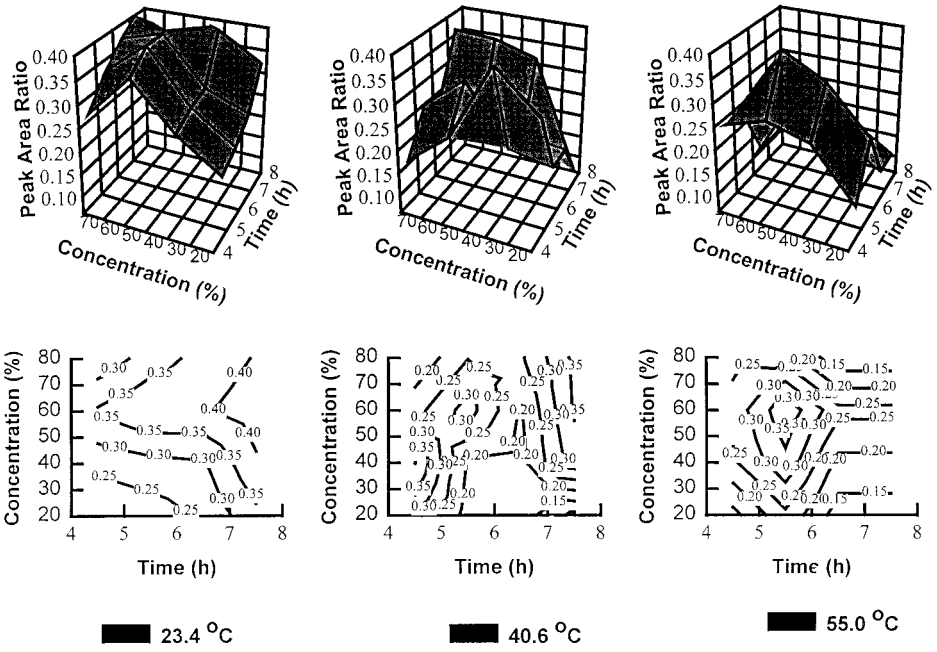

Figure 4. Influence of extraction time and solvent concentration (% ethanol in acetone) on the peak area ratio of hypericin at different extraction temperatures. The three-dimensional graphs on the top part of the figure were plotted by using the experimental data. The contour plots on the bottom part of figure were based on the response surface models.

II experiment. If hyperforin were present in the sample, it would be extracted using this solvent system (Liu et al., 2000).

The extraction time was another important parameter for the extraction procedure. In the phase I experiment, *H. perforatum* powders were extracted with 50% ethanol−acetone for 15, 30, 45, 60, 240, 480, 720, and 1440 min. The results showed (Figure 2) that the extraction efficiencies significantly increased when the extraction time increased from 15 to 480 min. After 480 min, increasing the extraction time did not improve the extraction efficiency significantly. Beyond 480 min, both the extractable material weight and selected peak area ratios actually decreased a little. This might be caused by decomposition of some compounds during the long extraction time. These results indicated that the best extraction time would be between 240 and 480 min (between 4 and 8 h).

Phase II. Effects of Temperature. The effect of temperature on the extraction efficiency in this experiment was not simple because the extraction matrix was complicated. When the experimental data were fitted to the response surface model with temperature, time, and concentration as independent variables, the stationary points for all response variables, except P6 and P7, were saddle points. Although P6 and P7 had

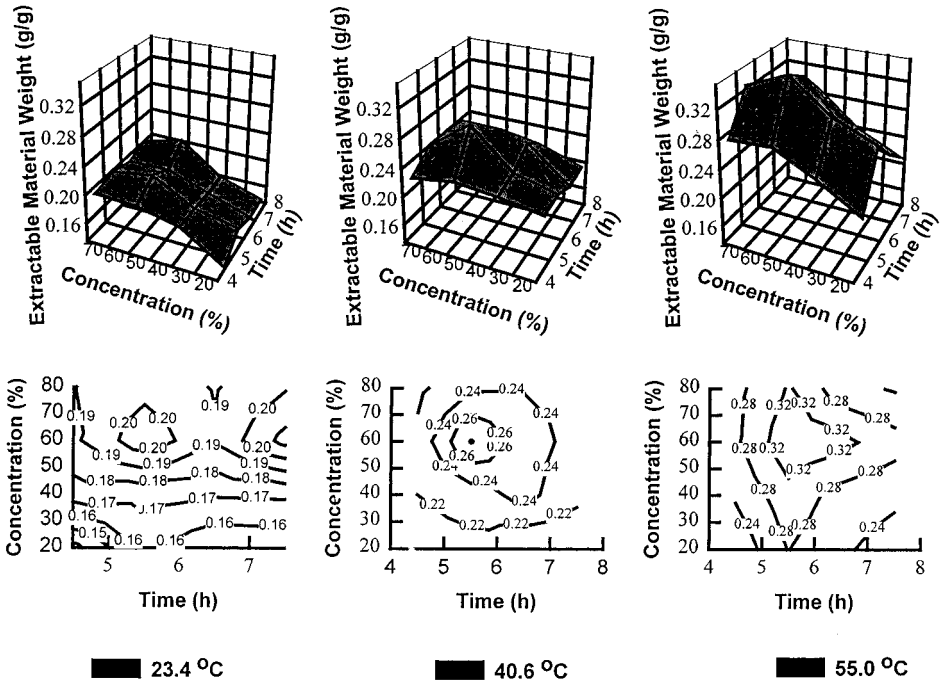

Figure 5. Influence of extraction time and solvent concentration (% ethanol in acetone) on the extractable material weight at different extraction temperatures. The three-dimensional graphs on the top part of the figure were plotted by using the experimental data. The contour plots on the bottom part of figure were based on the response surface models.

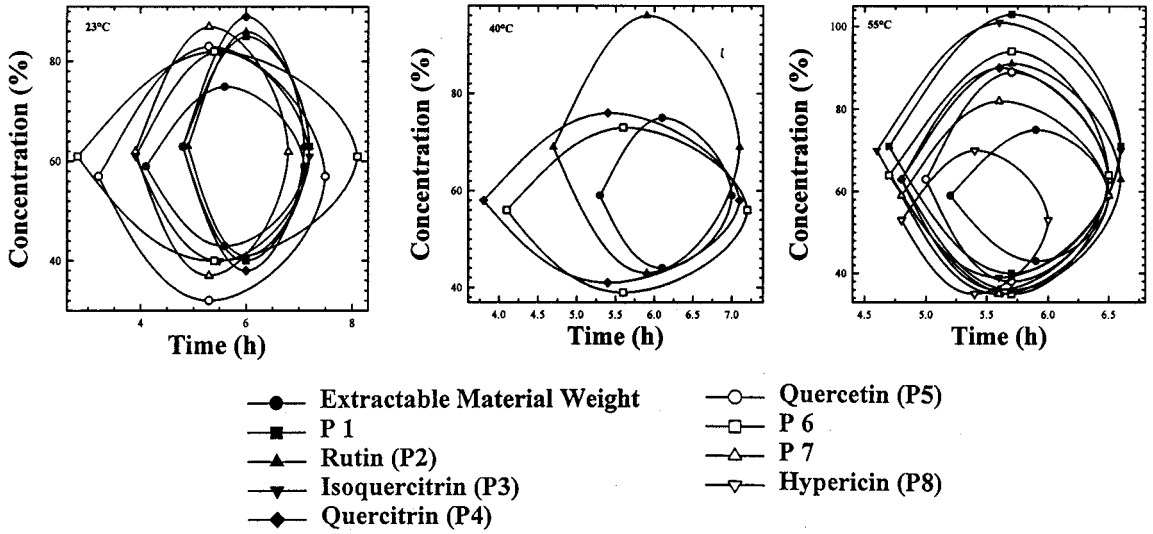

Figure 6. Ninety-five percent confidence ranges of each stationary point of the variables at three different temperatures.

maximum stationary points, their critical values (Table 3) lay outside the Phase II experimental range. When the experimental data were fitted to the model at each temperature separately, most of the response variables had maximum stationary points and their critical values were in the experiment range (Table 3). In the experimental condition, when the extraction temperature increased from 23 to 40 °C and then to 55 °C, the extraction efficiencies for all selected peak areas, except hypericin, increased but without linear correlation. When the temperature increased from 23 to 40 °C, the efficiencies only increased slightly, while from 40 to 55 °C, they increased sharply (Table 3 and Figures 3 and 4). The temperature above 55 °C was not tested in this experiment because the boiling point of acetone, a component of the solvent, is 56 °C. The total extractable material weight was another index to demonstrate the overall extraction efficiency. It increased with increasing temperature from 23 to 40 °C and from 40 to 55 °C (Figure 5).

Phase II. Effects of Extraction Time and Solvent Concentration. Phase I experimental results showed that optimum ranges of time and solvent concentration for extraction were 4−8 h and 100% ethanol to 100% acetone, respectively. To achieve the maximum extraction efficiency, four times and four temperatures with equal intervals within these ranges were used and the results were fitted to response surface models at three different temperatures. All response variables, except quercetin (P5) at 40 °C and hypericin (P8) at 23 and 40 °C, gave maximum stationary points, and most critical values of time and concentration were within the phase II testing range (Table 3). Although the critical values of time and concentration for different response vari-

Table 4. Verification of the Quadratic Response Surface Model[a]

		HPLC peak area ratio per gram of sample weight[c]							
	EMW[b] (g/g)	P1	P2	P3	P4	P5	P6	P7	P8
Solvent, 60% Ethanol; Extraction Time, 5.7 h; Extraction Temperature, 23 °C									
P value	0.0001	0.0002	0.0002	0.0006	0.0029	0.066[f]	0.0022	0.0005	0.0033
R-square	0.54	0.58	0.59	0.55	0.48	0.31	0.49	0.55	0.48
low 95% mean[d]	0.229	0.95	3.42	0.70	0.30	0.08	0.35	1.28	0.30
$value_{pred}$[d]	0.246	1.04	3.70	0.78	0.32	0.09	0.40	1.41	0.35
high 95% mean[d]	0.263	1.14	3.97	0.85	0.35	0.10	0.44	1.55	0.40
$value_{meas}$[e]	0.232	1.02	4.23[g]	0.58[g]	0.28[g]	0.09	0.445[g]	1.51	0.36
Solvent, 60% Ethanol; Extraction Time, 5.8 h; Extraction Temperature, 40 °C									
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
R-square	0.64	0.61	0.63	0.64	0.62	0.72	0.65	0.77	0.62
low 95% mean[d]	0.246	0.99	3.66	0.69	0.34	0.11	0.51	1.58	0.21
$value_{pred}$[d]	0.252	1.08	4.00	0.76	0.37	0.12	0.55	1.68	0.25
high 95% mean[d]	0.258	1.18	4.35	0.82	0.40	0.13	0.59	1.79	0.30
$value_{meas}$[e]	0.245	1.18	4.18	0.82	0.35	0.12	0.53	1.61	0.27
Solvent, 60% Ethanol; Extraction Time, 5.6 h; Extraction Temperature, 55 °C									
P value	0.0001	0.0004	0.0006	0.0029	0.0005	0.0001	0.0004	0.0002	0.0001
R-square	0.69	0.59	0.58	0.51	0.58	0.69	0.59	0.62	0.79
low 95% mean[d]	0.312	1.58	5.75	1.04	0.48	0.20	0.72	2.02	0.31
$value_{pred}$[d]	0.323	1.88	6.80	1.28	0.57	0.24	0.85	2.28	0.35
high 95% mean[d]	0.334	2.18	7.84	1.51	0.66	0.27	0.99	2.55	0.39
$value_{meas}$[e]	0.315	2.05	6.14	1.31	0.55	0.24	0.82	2.39	0.34

[a] $Y = a_1 + a_2X_1 + a_3X_2 + a_4X_1^2 + a_5X_1X_2 + a_6X_2^2$. Y = dependent variable, represents EMW (extractable material weight) or peak area ratio of P1, rutin, isoquercitrin, quercitrin, quercetin, P6, P7, or hypericin. X_1 and X_2 = independent variables, represent time and concentration, respectively. [b] EWM = extractable material weight, g/g. [c] Peak area of the sample component as the relative response to the internal standard: (P1, P6, and P7) unknown; (P2) rutin; (P3) isoquercitrin; (P4) quercitrin; (P5) quercetin; (P8) hypericin. [d] The value was calculated for each tested point on the basis of quadratic model. [e] The value was the average of four measurements for each tested point. [f] The quadratic model was not significant at the 95% level. [g] The measured value was out of the 95% confidence range from the predicted value.

ables differed, their 95% confidence ranges were overlaid (Figure 6). Considering all these variables, on the basis of the 95% confidence range, it was concluded that the optimum ranges of time and concentration were 5.0−6.7 h and 44−74% at 23 °C, 5.4−6.9 h and 45−72% at 40 °C, and 5.3−5.9 h and 44−69% at 55 °C, respectively. All variables, except hypericin, had the highest extraction efficiencies at 55 °C.

Phase III. Verification of the Quadratic Response Surface Model. The models for all response variables, except quercetin (P5) at 23 °C, were statistically significant by the *F* test at the 95% level. For most variables, the models fitted better at 40 or 55 °C than that at 23 °C (Table 4).

For verification of the accuracy of the model, a point within the optimum time and concentration range at each temperature was chosen for an extraction test. Most measured values of the response variables were within the 95% confidence range of predicted values based on quadratic response surface models (Table 4), except peak area ratios of rutin (P2), isoquercitrin (P3), quercitrin (P4), and P6 at 23 °C.

Conclusion. The response surface method was effective in optimizing the extraction conditions of *H. perforatum*. Prediction models of different response variables including extractable material weight and peak area ratios of P1, rutin (P2), isoquericitrin (P3), quercitrin (P4), quercetin (P5), P6, P7, and hypericin (P8) correlated with extraction time and solvent concentration significantly. Although the extraction temperature was not included in these models, the temperature significantly affected the extraction efficiency. For most response variables, the temperature had a positive effect. At different temperatures, they had different optimum extraction time and solvent concentration ranges. The optimum extraction conditions were 44−69% ethanol in acetone as solvent, 5.3−5.9 h, and 55 °C water−bath shaker.

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