A Method for Quantitative Determination of Furanocoumarins in Capsules and Tablets of Phytochemical Preparations

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A method for sample preparation and analysis by high-performance liquid chromatography with UV detection (HPLC-UV) was developed for analysis of psoralen, bergapten and 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one in capsules and tablets employed in Brazil for certain illnesses. The linearity, accuracy, the inter- and intra-day precision of the procedure were evaluated. Analytical curves for furanocoumarins were linear in the range of 1.0—50.0 μg/ml. The recoveries of the furanocoumarins in the products analyzed were 97.3—99.5%, and the percent coefficient of variation for the quantitative analysis of the furanocoumarins in the analyses was under 5%. For inter-equipment study gas chromatography (GC) was employed.

Key words liquid chromatography; quantification; furanocoumarin; psoralen

Furanocoumarins are known as photoreactive compounds and are increasingly used for photochemotherapic dermatologic treatments of diseases such as vitiligo, psoriasis, mycosis fungoides, atopic eczema and alopecia areata, among others.[1,2] The furanocoumarins have been used in some pharmaceutical and cosmetic products because of their UV light absorbing properties.[3] In photochemotherapy psoralens are often employed orally or topically, associated with Ultraviolet A (UVA) irradiation. The combination of these cited compounds with UVA irradiation is known as PUVA therapy (psoralens plus UVA irradiation).[4—7]

The biological activity of these compounds is attributable to their ability to intercalate into DNA, where they are able to form monoadducts and crosslinks in the presence of longwave UV light.[8] On the other hand, use of psoralens in medicine has been associated with higher incidence of skin cancer,[8—10] and several studies have demonstrated that the psoralens are carcinogenic, mutagenic and photodermatitic.[11,12] Linear psoralens cause phototoxicity.[13,14] Usually, the amount of the compounds psoralen and bergapten is used as an index of the phototoxic activity of a plant.[15]

In preliminary analysis psoralen, bergaten and 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one were identified in samples analyzed in this study. The capsules and tablets employed are indicated, according to their technical information, for the treatment of menstrual irregularities, abdominal and menstrual cramps, vaginal discharge, daily pre-menstrual tension, disturbances related to the menopause, inflammations of the lymphatic nodes or vitiligo.

For most of the phytomedicines used in Brazil there is no information on their exact composition in relation to the substances they contain or on the recommended dosages, imposing serious risks to public health. The samples analyzed showed the presence of *Dorstenia* species and other plants. *Dorstenia* species are known for their ability to synthesize linear and angular furanocoumarins.[15]

HPLC equipment is widely disseminated in Brazil. We have developed an analytical procedure suitable for sample preparation and HPLC-UV analysis of the three furanocoumarins mentioned earlier in phytomedicines (capsules and tablets). The HPLC technique has been shown to be a very efficient system for separating complex mixtures like phytomedicines. HPLC methods have been reported for the determination of psoralens in callus cultures, vitro culture, serum, dermis, plants, citrus, essential oils and phytomedicines, but only the most recent published methods report assay validation.[6,15—24] Liquid and gas chromatographic methods have shown the determination of furanocoumarins in phytomedicines,[15,25] however, there are no descriptions in the literature about sample preparation or analysis of psoralen (P), bergapten (G) or 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one (DT) (Fig. 1) present in phytochemical preparations (capsules and tablets) employed in Brazil.

The validation of the procedure was carried out according to the International Conference on Harmonization guidelines.[26,27] The sensitivity, specificity, linearity, accuracy, the inter- and intra-day precisions and inter-equipment variability of the assay method were evaluated. Efficiency of the analytical procedure was assessed through calculation of recovery values.

Experimental

Products Three capsules and two tablets labeled as capsules A, B and C and tablets D and E from a different manufacturer (three lots each) were used for method development and validation. These products contain the follow compositions: A (*Dorstenia multiformes–Plumeria lancifolia*), B (*Dorstenia multiformis–Plumeria lancifolia–Cereus jamacaru–Erythrina mulungu*), C (*Dorstenia brasiliensis*), D and E (natural product).

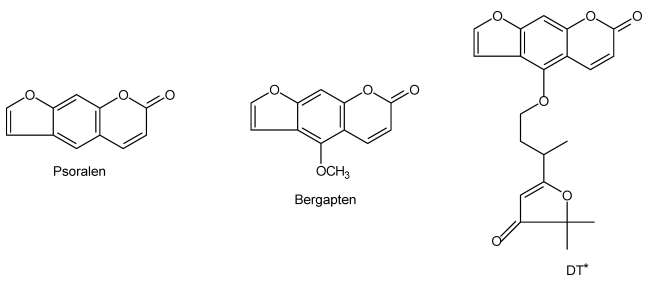

Fig. 1. Chemical Structures of the Furanocoumarins

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Chemicals Spectroscopy-grade acetonitrile, methanol and chloroform were purchased from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore). Furanocoumarin standards were available from a collection purified by chromatography and recrystallization at our laboratory. The purity of the standards was confirmed by mass and hydrogen NMR spectra and with published values of HPLC retention times. P, G and DT standards were obtained with a purity of 98.7%, 98.9% and 97.8%, respectively Stock mixtures of these standards were made from the individual solutions in methanol and used as external standards.

HPLC Conditions The analyses were performed on a Shimadzu liquid chromatograph with a ternary solvent delivery system—Model LC-6AD, combined with a UV–Vis detector—Model SPD-6AV (Shimadzu Co., Kyoto, Japan), and a Rheodyne injection valve fitted with a 100-μl sample loop. A microcomputer equipped with Microquimica-MQI18PCA software was used for recording chromatograms and measuring peak areas. HPLC separation of the psoralens was performed using a Shimadzu octadecyl Shim-pack CLC-ODS (4.6 mm i.d.×25 cm long and 5 μm particle diameter) reversed-phase column with a small pre-column (4.6 mm i.d.×2.5 cm long) containing the same packing, used to protect the analytical column. Before use, the solvents were filtered through a 0.45-μm HV filter (Millipore), then degassed for 20 min in an ultrasonic bath. Elution was performed with acetronitrile–water (55 : 45, v/v) at a flow-rate of 1.0 ml/min. Aliquots of 10 μl were injected with a 25 μl Hamilton syringe. After determination the column was cleaned for 10 min with the same system solvent and flow-rate. Detection of the peaks was recorded at 223 nm. All chromatographic analyses were performed at 22 °C.

GC-MS Conditions The analyses were performed on a Shimadzu Co. model QP 500, with electron impact ionization (70 eV), coupled to Shimadzu GC-17 gas chromatograph. The psoralens were separated using a fused silica LM-5 (15 m×0.2 i.d., film thickness 0.2 μm) capillary. The injection temperature was 280 °C. Column temperature was programmed from 150 to 240 °C, with a linear increase of 10 °C/min, then held for 20 min. The detector temperature was 280 °C, the injection split ratio was 1 : 20. Aliquots of 1 μl were injected with a 10-μl Hamilton syringe.

Sample Preparation Several forms for extraction of P, G and DT from the capsules and tablets were tested, such as changing the kind and volume of solvent and the period in sonication and in centrifugation (5000 rpm). The optimized procedure found was: each sample (100 mg) was extracted with 10 ml of chloroform–methanol (3 : 7, v/v) in sonication for 15 min and then was centrifuged for 10 min. The residue was reextracted with the same solvent for the same times in sonication and centrifugation. The two extracts were combined and the solvent was evaporated to dryness in a stream of nitrogen. This residue was dissolved in 2 ml of methanol, filtered through a 0.45 μm Millex filter and the solution was diluted in methanol in a volumetric flask (5 ml or 10 ml) in order to be analyzed by HPLC and GC-MS.

Determination of the Detection and Quantification Limits The detection limits were determined by injecting (n=5) solutions of standards of known concentration (10 μl each), and decreasing the concentrations of the samples until a peak was detected with three times the signal/noise ratio. The corresponding concentration was considered to be the minimal detectable concentration. The quantification limits were determined by the same methodology and the quantification limit was considered the chromatography peak having ten times the signal/noise ratio.

Extraction Recovery The extraction efficiency (recovery) was determined from samples of each capsule and tablet spiked with standards, corresponding to low, medium and high concentrations. The spiked samples were submitted to the same procedure as described in the sample preparation.

Analytical Curves Estimation of the content of P, G and DT in the samples was performed by external calibration. The compounds in the study were dissolved separately in spectroscopy-grade methanol in order to obtain stock solutions, which were appropriately diluted to concentrations ranging from 1—50 μg/ml for each of the substances. Aliquots of 10 dilutions for each standard were analyzed by HPLC and GC-MS, with each determination being carried out five times. For each standard was obtained a corresponding chromatogram and constructed a graphic plot of the means of areas made against the concentration of each furanocoumarin. Linear least squares regression of the peak areas as a function of the concentrations was performed to determine correlation coefficients. The equation parameters (slope and intercept) of each standard curve were used to obtain concentration values for quality control samples and unknown samples of capsules and tablets. Specimens with an analyte concentration exceeding the analytical curve were reassayed upon appropriate dilution of the samples.

Linearity The linearity of the assayed method was evaluated crossing the range of the analytical procedure. This was done by analyzing each sample (capsule and tablet) spiked with a known amount of the analytes at low, medium and high concentrations. Aliquots (10 μl) were analyzed by HPLC as described above. Each determination was carried out five times. For each spiked sample the corresponding chromatograms were obtained and a plot of the average of the areas against their concentrations was constructed. Linear least squares regression was performed to determine the correlation coefficients.

Accuracy and Precision The accuracy of the assayed method was evaluated by performing replicate analyses against an analytical calibration curve and calculating the mean percent differences between the theoretical values and the measured values. The accuracy values in intra- and inter-day variation studies using HPLC at low, medium and high concentrations of standards were evaluated for all the capsules and tablets. Precision of the method is expressed as the percentage of the coefficient of variation (CV) of replicate measurements, and was tested for both intra-day and inter-day repeatability in the capsules and tablets by HPLC. The intra-day variability of the method was determined by three different analyses (n=5) for each sample of capsule and tablet with the addition of known amounts of analyte at low, medium and high concentrations. The inter-day variability was verified by the same procedure on three different days (n=5). The GC-MS was employed for inter-equipment variability.

Stability Study The stability of the working standard solutions was tested at 22 °C (working temperature), 4 °C and −20 °C (storage temperatures).The stability of P, G and DT in the samples was evaluated during all the storage steps (*i.e.* at room temperature, at 4 °C and at −20 °C). Spiked samples were analyzed against the analytical calibration curves immediately after preparation (reference values) and after storage. Stability was defined as being less than 2% loss of the initial drug concentration in the stated time.

Specificity To evaluate the specificity of the method, two other furocoumarins (isopimpinellin and isobergapten), usually present in the genus *Dorstenia*, were assayed by the same procedures using HPLC and GC-MS, and the retention times of these compounds were compared with those of P, G and DT in the samples.

Results and Discussion

Products A and B indicate the presence of coumarins in their technical information, but none of the products studied had information about furanocoumarins in their compositions. A number of preliminary sample preparations and HPLC experiments employing capsules and tablets were performed to establish optimal conditions for sample preparation and HPLC analysis of P, G and DT.

HPLC analysis showed baseline separation for the compounds of interest, which could be analyzed in a satisfactory period of time, less than 12 min (psoralen 6.15±0.04 min, bergapten 7.45±0.03 min and 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one 11.44±0.03 min) (Figs. 2—4). The intervals, where the compounds eluted, were free of interference in all

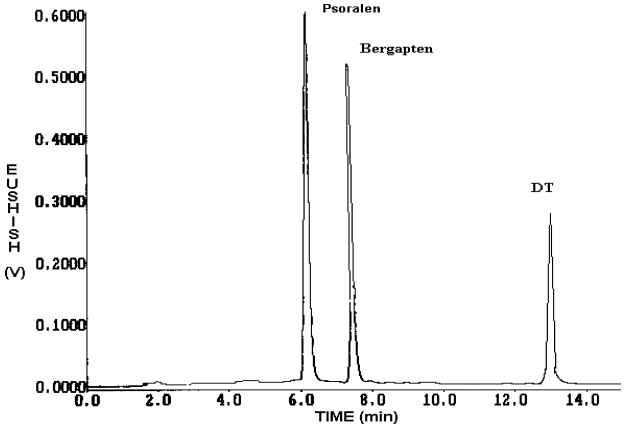

Fig. 2. Chromatogram of a Standard Analysis by HPLC
For chromatographic conditions see Experimental.

samples tested.

GC-MS analysis also showed baseline separation for the compounds of interest, which could be analyzed in a satisfactory time interval, less than 17 min (psoralen 4.32±0.05 min, bergapten 6.33±0.04 min and 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one 16.45±0.05 min) (Fig. 4).

The identification of P, G and DT in the capsules and tablets was made by comparison of their retention time with those of authentic standards and by standards addition in the samples analyzed by HPLC-UV and GC-MS.

No changes in the three furanocoumarins were detected in working solutions after 24 h at 22 °C, 2 months at 4 °C and 6 months of storage at −20 °C. All three were stable in the samples after 24 h at 22 °C, one month of storage at 4 °C and 6 months of storage at −20 °C. Thus this validated method for the assay of psoralens can be regarded as indicating stability of the solutions.

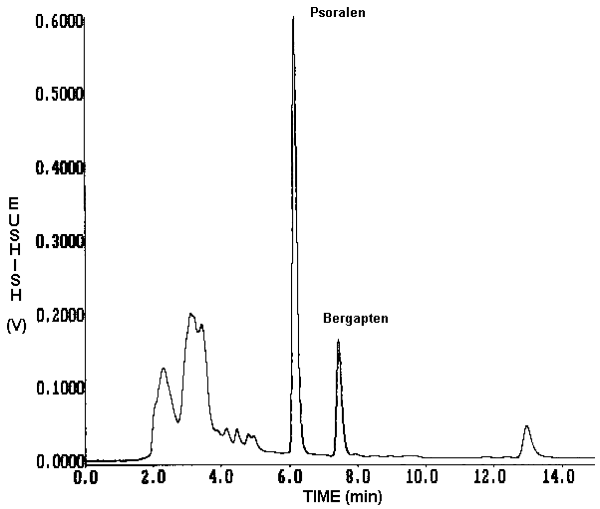

Fig. 3. Chromatogram of a Capsule A Analysis by HPLC
For chromatographic conditions see Experimental.

The calibration curves were determined by linear regression, and were linear in the range of 1.00—50.00 μg/ml for the three by both HPLC and GC-MS (Tables 1, 2). Average standard errors for the peak areas of replicate injections (n=5) were smaller than 1% showing good repeatability of the calibration curve.

The linearity of the method was determined by linear regression. The analysis of samples spiked with known amounts of analyte demonstrated that the response was proportional to the concentrations of the samples with the determination coefficient r^2=0.9998 for the linear range of the analytical calibration curves for samples of capsules and tablets.

Detection limits were 0.030 μg/ml for P, 0.070 μg/ml for G and 0.15 μg/ml for DT and quantification limits were 0.10 μg/ml for P, 0.23 μg/ml for G and 0.50 μg/ml for DT by HPLC. Detection limits were 0.10 μg/ml for P, 0.090 μg/ml for G and 0.24 μg/ml for DT and quantification limits were 0.33 μg/ml for P, 0.30 μg/ml for G and 0.80 μg/ml for DT by GC-MS.

The recovery results showed that the procedure used is good for the extraction of the substances of interest in the capsules and tablets (Table 3).

The accuracy values were less than 6% (Tables 4, 5). Regarding the precision of the assay, intra- and inter-day CVs were less than ±5%. In this work the precision of the method

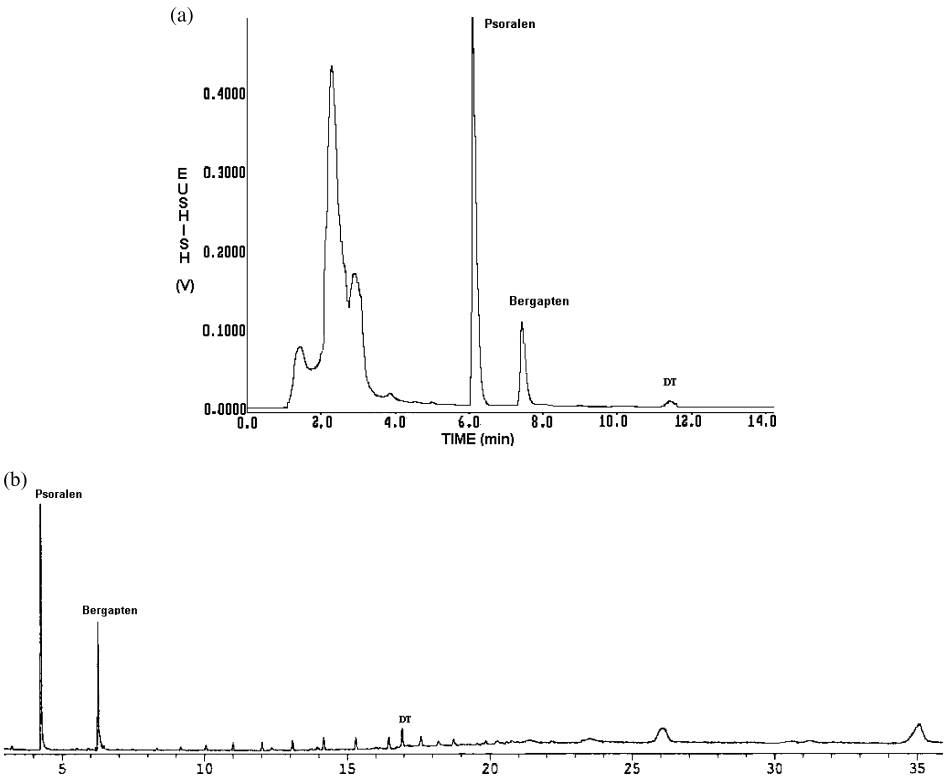

Fig. 4. Chromatogram of a Capsule B Analysis by HPLC (a) and by GC-MS (b)
For chromatographic conditions see Experimental.

Table 1. Regression Data of Analytical Calibration Curves for Quantitative Determination of the Substances by HPLC

	Substances		
	Psoralen	Bergapten	DT
LR (μg/ml)	1.00—50.00	1.00—50.00	1.00—50.00
b	0.21645	0.23119	0.34576
a	0.03337	0.02137	0.03236
Sa	0.02345	0.02101	0.02905
Sb	0.00132	0.00129	0.00157
r	0.9998	0.9999	0.9997
n	10	10	10

DT: 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one. LR: linear range, *b*: slope, *a*: intercept, Sb: standard deviation of the slope, Sa: standard deviation of the intercept, *r*: correlation coefficient, *n*: number of samples. Linear regression, formula: $y=a+bx$, where y=peak areas ratio, x=concentration ($\mu g\,ml^{-1}$), a=intercept and b=slope.

Table 2. Regression Data of Analytical Calibration Curves for Quantitative Determination of the Substances by GC-MS

	Substances		
	Psoralen	Bergapten	DT
LR (μg/ml)	1.00—50.00	1.00—50.00	1.00—50.00
b	0.35345	0.37781	0.49872
a	0.04139	0.03901	0.04789
Sa	0.02678	0.02341	0.02659
Sb	0.00229	0.00237	0.00217
r	0.9998	0.9998	0.9997
n	10	10	10

DT: 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one. LR: linear range, *b*: slope, *a*: intercept, Sb: standard deviation of the slope, Sa: standard deviation of the intercept, *r*: correlation coefficient, *n*: number of samples. Linear regression, formula: $y=a+bx$, where y=peak area ratio, x=concentration ($\mu g\,ml^{-1}$), a=intercept and b=slope.

Table 3. Recovery of the Furanocoumarins in Capsules and Tablets Samples A—E ($n=5$)

Conc. added (μg/ml)	Psoralen (%) (mean±S.D.)				
	A	B	C	D	E
1	97.33±1.36	97.99±0.94	97.34±0.99	97.45±1.16	97.89±0.87
20	98.79±0.85	99.14±1.37	98,98±0.96	98.96±0.78	99.44±1.11
40	99.03±0.97	99.05±0.77	99.47±1.27	99.29±0.91	99.27±0.80

Conc. added (μg/ml)	Bergapten (%) (mean±S.D.)				
	A	B	C	D	E
1	98.13±0.81	98.87±1.12	98.01±1.19	97.89±1.10	98.67±0.83
20	99.43±0.71	99.46±0.91	99.26±0.73	99.02±0.86	99.29±1.11
40	99.01±0.67	99.39±0.80	98.11±1.03	99.44±0.93	99.33±0.76

Conc. added (μg/ml)	DT (%) (mean±S.D.)				
	A	B	C	D	E
1	97.45±1.28	97.88±0.99	97.67±0.87	97.74±1.25	98.68±1.03
20	98.99±0.73	99.35±1.27	99.01±1.13	98.76±0.89	99.50±1.19
40	99.14±1.02	99.13±0.97	98.00±1.22	99.36±1.27	99.55±0.82

Conc., concentration; S.D., standard deviation.

Table 4. Intra-day Accuracy and Precision of HPLC Method for Determination of the Furanocoumarins in Capsules and Tablets Samples A—E ($n=5$, for Each Sample)

C added (μg/ml)	Psoralen (μg/ml) (mean±S.D.)			Bergapten (μg/ml) (mean±S.D.)			DT (μg/ml) (mean±S.D.)		
	C found (μg/ml) (mean±S.D.)	Ac (%)	CV (%)	C found (μg/ml) (mean±S.D.)	Ac (%)	CV (%)	C found (μg/ml) (mean±S.D.)	Ac (%)	CV (%)
1	1.03±0.05	3.00	4.85	1.01±0.04	1.00	3.96	1.03±0.05	3.00	3.96
20	19.51±0.53	2.45	2.72	20.02±0.47	0.10	2.35	20.08±0.57	0.40	2.84
40	39.32±0.91	1.70	2.31	39.51±0.85	1.23	2.15	39.43±0.99	0.14	2.51

C, concentration; CV, coefficient of variation; Ac, accuracy; S.D., standard deviations.

was tested for both intra-day and inter-day repeatability and the variability of the method was determined at low, medium and high concentrations. The results are shown in Tables 4 and 5. These data indicate that the method is reproducible within the same day and on three different days.

The GC-MS showed higher detection and quantification limits, while the HPLC procedure using ultraviolet detection presents lower quantification and detection limits. Both methods can be used to analyze psoralens in capsules and tablets.

Variance analyses revealed no statistical differences between data obtained by HPLC and GC at a 5% level signifi-

Table 5. Inter-day Accuracy and Precision of HPLC Method for Determination of the Furanocoumarins in Capsules and Tablets Samples A—E ($n=5$, Each Sample)

C added (μg/ml)	Psoralen (μg/ml) (mean±S.D.)			Bergapten (μg/ml) (mean±S.D.)			DT (μg/ml) (mean±S.D.)		
	C found (μg/ml) (mean±S.D.)	Ac (%)	CV (%)	C found (μg/ml) (mean±S.D.)	Ac (%)	CV (%)	C found (μg/ml) (mean±S.D.)	Ac (%)	CV (%)
1	1.05±0.05	5.00	4.76	1.02±0.05	2.00	4.90	1.03±0.05	3.00	3.96
20	19.78±0.59	1.10	3.01	20.06±0.51	0.30	2.54	20.10±0.42	0.50	4.20
40	39.50±0.82	1.25	2.08	39.63±0.79	0.93	1.99	39.50±0.87	1.25	2.20

C, concentration; CV, coefficient of variation; Ac, accuracy; S.D., standard deviations.

Table 6. Contents (μg/100 mg of Capsule or Tablet) (Mean±S.D.) of the Furanocoumarins Employing the HPLC Method

Products	Psoralen	Bergapten	DT
A1	250±1.4	83±1.3	—
A2	249±1.3	85±0.9	—
A3	250±1.4	80±1.2	—
B1	210±2.2	60±1.7	10±0.3
B2	213±2.2	63±2.1	12±0.3
B3	210±2.0	67±1.4	14±0.4
C1	172±2.6	75±1.9	—
C2	70±1.1	72±2.5	—
C3	237±2.4	94±3.3	—
D1	225±2.2	55±1.7	11±0.2
D2	232±2.2	56±2.1	13±0.3
D3	222±2.0	53±1.4	12±0.4
E1	—	—	—
E2	—	—	—
E3	—	—	—

S.D., standard deviations; S.D. of five determinations.

cance.

In this study, concentrations between 0.35 mg and 1.25 mg were found for psoralen, 0.26—0.47 mg for bergapten and 0.050—0.070 mg for 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one in both capsules and tablets (Table 6). The dose recommended by manufacturers for the phytomedicines A—E is 3 capsules or tablets per day. Thus the dose consumed per person of the analyzed sample would be 1.05—3.75 mg/d (7.35—26.25 mg/week) of psoralen, 0.80—1.40 mg/d (5.60—9.80 mg/week) of bergapten and 0.15—0.21 mg/d (1.05—1.47 mg/week) of 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one. These doses are sub-therapeutic for vitiligo and psoriasis. However, the lack of knowledge of the presence of furanocoumarins in the phytochemical preparations offers risks to the public health, since they are not adversed about the potencialization of the effects carcinogenicity, mutagenicity or phototoxicity of psoralens after exposure to the sun. Therefore, it is important also know the levels of psoralens in capsules and tablets consumed by humans. For health problems (menstrual irregularities, abdominal and menstrual cramps, vaginal discharge, daily pre-menstrual tension, disturbances related to the menopause, inflammations of the lymphatic nodes) the consumption of psoralens is not recommended in the literature.

For capsules A and B there were no discrepancies in the concentrations of psoralens among the lots analyzed. Capsule C showed discrepancies of 150—240% in psoralen and 4—30% in bergapten among the contents of the three lots (Table 6). Presence of psoralen was not detected in tablet E (Table 6). In the three lots analyzed 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7one was detected in samples B and D (Table 6).

Conclusion

A methodology for sample preparation and HPLC analysis of the capsules and tablets was developed for the simultaneous determination of psoralen, bergapten and 5-[3-(4,5-dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7*H*-furo[3-2-*g*][1]benzopyran-7-one, providing a method for their quality control. This method does not require any tedious procedures, and validation experiments showed good precision and accuracy with coefficients of variation and relative errors less than ±5%.

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References and Notes

1) Lehr G. J., Barry T. L., Franolic J. D., Petzinger G., Scheiner P., *J. Pharm. Biomed. Anal.*, **33**, 627—637 (2003).
2) Bettero A., Benassi C. A., *J. Chromatogr.* A, **280**, 167—171 (1983).
3) Järvenpää E. P., Jestoi M. N., Huopalahti R., *Phytochem. Anal.*, **8**, 250—256 (1997).
4) Zanini A. C., Basile A. C., Follador W., Oga S., "Guia de Medicamentos," Iepx, São Roque, 1997, pp. 905—906.
5) Makki S., Muret P., Said A. M., Bassignot P., Humbert P., Agache P., Millet J., *Int. J. Pharm.*, **133**, 245—252 (1996).
6) Cardoso C. A. L., Vilegas W., Honda N. K., *J. Pharm. Biomed. Anal.*, **22**, 203—214 (2000).
7) Steiner D., *Cosmetics & Toiletries*, **10**, 33 (1998).
8) Chimichi S., Boccalini M., Cosimelli B., Viola G., Vedaldi D., Dall'Acqua F., *Tetrahedron*, **58**, 4859—4863 (2002).
9) Nigg H. N., Strandberg J. O., Beier R. C., Petersen H. D., Harrison J. M., *J. Agric. Food Chem.*, **45**, 1430—1436 (1997).
10) Kreimer-Erlacher H., Seidl H., Bäck B., Cerroni L., Kerl H., Wolf P., *J. Invest. Dermatol.*, **120**, 676—682 (2003).
11) Chouchi D., Barth D., *J. Chromatogr. A*, **672**, 177—183 (1994).
12) Vilegas J. H. Y., Lanças F. M., Vilegas W., Pozetti G. L., *J. Braz. Chem. Soc.*, **8**, 529—535 (1997).
13) Franke K., Porzel A., Masaoud M., Adam G., Schimidt J., *Phytochemistry*, **56**, 611—621 (2001).
14) Dercks W., Trumble J. T., Winter C., *J. Chem. Ecol.*, **16**, 443—449 (1990).
15) Cardoso C. A. L., Vilegas W., Barison A., Honda N. K., *J. Agric. Food Chem.*, **50**, 1465—1469 (2002).
16) Bonaccorsi I., Dugo G., McNair H. M., Dugo P., *Ital. J. Food Sci.*, **12**, 485—487 (2000).
17) Dugo P., Mondello L., Dugo L., Stancanelli R., Dugo G., *J. Pharm. Biomed. Anal.*, **24**, 147—154 (2000).
18) Ekiert H., Gomolka E., *Pharmazie*, **55**, 684—687 (2000).
19) Ekiert H., Gomolka E., *Pharmazie*, **55**, 618—620 (2000).
20) Verzera A., Dugo P., Mondello L., Trozzi A., Cotroneo A., *Ital. J. Food Sci.*, **11**, 361—366 (1999).

21) Bonaccorsi I. L., McNair H. M., Brunner L. A., Dugo P., Dugo G., *J. Agric. Food Chem.*, **47**, 4237—4239 (1999).
22) Markowski W., Czapinska S. L., Chem K. L., *Anal-Warsaw*, **42**, 353—357 (1997).
23) Wang L., Tso M., *J. Pharm. Biomed. Anal.*, **30**, 593—600 (2002).
24) Leveque N., Muret P., Mary S., Bérard M., Makki S., Kantelip J. P., Humbert P., *J. Chromatogr. B.*, **780**, 119—127 (2002).
25) Cardoso C. A. L., Honda N. K., Barison A., *J. Pharm. Biomed. Anal.*, **27**, 217—224 (2002).
26) Validation of Analytical Procedures: Methodology, International Conference on the Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use, *Q2B*.
27) Text on Validation of Analytical Procedures, International Conference on the Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use, *Q2A*.