J. Pharm. Pharmacol. 1999, 51: 193–200
Received March 31, 1998
Accepted October 7, 1998

© 1999 J. Pharm. Pharmacol.

Isolation, Purity Analysis and Stability of Hyperforin as a Standard Material from *Hypericum perforatum* L.

H. C. J. ORTH, C. RENTEL* AND P. C. SCHMIDT

*Department of Pharmaceutical Technology, Auf der Morgenstelle 8 and *Department of Organic Chemistry, Auf der Morgenstelle 18, Eberhard-Karls University, D-72076 Tübingen, Germany*

Abstract

In 1996 131·5 million daily doses of preparations containing extracts of *Hypericum perforatum* L. were prescribed in Germany for treating mild to moderately severe depressive disorders. New pharmacological and clinical results focus on hyperforin as the main active ingredient of the drug.

Hyperforin ($C_{35}H_{52}O_4$) is one of the main components (2–4%) of the dried herb *Hypericum perforatum* L. It was isolated after six consecutive steps: extraction of deep-frozen blossoms (−20°C) with *n*-hexane by means of an Ultra Turrax at room temperature; separation of lipophilic substances on a silica gel column; purification of the relevant fraction by preparative HPLC; evaporation of the mobile phase under reduced pressure; removal of the remaining water by freeze-drying; and storage of hyperforin at −20°C under nitrogen. The identity and purity of the isolated substance were determined by high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC) with diode-array and ultraviolet detection (DAD and UV), Fourier-transformed infrared (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, and liquid chromatography coupled with positive-ion electrospray-ionization tandem mass spectrometry (LC-ESI(+)-MS-MS). By use of these methods the purity of hyperforin was shown to be >99·9%. Peroxides present at each step of the isolation were detected by titration and by means of Merckoquant analytical peroxide test-strips. Elimination of the peroxides and stabilization of hyperforin was achieved by consistent protection from oxidation—the mobile phases were protected by use of ascorbic acid; evaporation and freeze-drying were performed under nitrogen; and the mobile phase used for preparative HPLC was sparged with helium. Stability testing was performed by HPLC—the samples were stored at −30°C in a normal atmosphere and at −20, 4, and 20°C in a normal atmosphere or under nitrogen. Results were compared with those obtained after storage under liquid nitrogen (−196°C).

Because of its high sensitivity to oxidation, hyperforin was more stable under nitrogen under all test conditions. There was no statistically significant difference between results obtained after 8 months at −20°C under nitrogen or at −30°C under a normal atmosphere and those from the reference sample stored under liquid nitrogen (−196°C). Despite this, because of the tendency of hyperforin to degrade, long-term storage at −70°C under nitrogen is recommended.

The genus Hypericum (Hypericaceae) comprises a large number of species widely distributed in many countries in Middle Europe, North America and Eastern Africa (Roth 1990). In Germany, the leaves, stems and roots of the species *Hypericum perforatum* L. have been widely reported as traditional remedies in folk medicine (Wichtl 1997). More recently an increasing number of experimental and clinical studies performed with commercial preparations of extracts of *Hypericum perforatum* L. (common St John's wort) have shown that these preparations are more effective than placebo for the treatment of mild to moderately severe depressive disorders (Bombardelli &

Correspondence: P. C. Schmidt, Department of Pharmaceutical Technology, Auf der Morgenstelle 8, Eberhard-Karls University, D-72076 Tübingen, Germany.

Morazzoni 1995; Linde et al 1996). The total amount prescribed is more than 131·5 million daily doses per year in Germany (Lohse & Mueller-Oerlinghausen 1997). Until 1996 preparations containing extracts of *Hypericum perforatum* L. were standardized on hypericin on the basis of an average daily dose of 0·2–1·0 mg, corresponding to 2–4 g of the crude drug (German Phytotherapeutic Monographs; in accordance with the recommendation of Commission E of the German Federal Public Health Department 1984, 1989).

The clinical relevance of the monoamine oxidase-inhibiting properties of hypericum extracts, with particular reference to the effect of hypericin (Suzuki et al 1984; Sparenberg et al 1993; Bladt & Wagner 1994; Butterweck et al 1997) is still a controversial subject. Although standardization of hypericum extracts by hypericin content might offer no guarantee of pharmacological equivalence, there is good correlation between the amount of this substance and those of the other main compounds such as flavonoids and biapigenine (Ostrowski 1988). Recent publications show that hyperforin might make an important contribution to the antidepressant activity of the extracts (Chatterjee et al 1998; Erdelmeier 1998; Laakmann et al 1998; Mueller et al 1998; Schellenberg et al 1998).

Focussing on hyperforin ($C_{35}H_{52}O_4$, 5-(2-methyl-1-oxo-propyl)-6-*exo*-methyl-1,3-*endo*,7-*exo*-tris-(3-methylbut-2-enyl)-6-*endo*-(4-methyl-pent-3-enyl)-bicyclo[3.3.1]-nonan-2,4,9-trione; Figure 1) for future use as a reference material, this study draws attention to a different approach to determine the quality of standard materials used to standardize extracts of *Hypericum perforatum* L. Although hyperforin is very susceptible to oxidation, it is one of the main compounds of the dried herb of *Hypericum perforatum* L.—the content is between 2 and 4% (Maisenbacher 1991). The objective of this study was to develop a scheme for isolation of hyperforin taking into consideration the purity, oxidation sensitivity and long-term stability of the isolated substance.

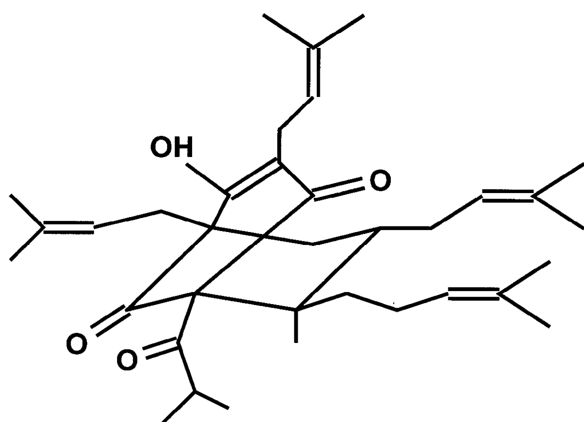

Figure 1. The structure of hyperforin ($C_{35}H_{52}O_4$).

Materials and Methods

Chemicals used

n-Hexane, *n*-heptane, *t*-butyl methyl ether, acetone, formic acid ethyl ester, methanol, toluene, acetonitrile, petroleum ether, twice-distilled water, chloroform, formic acid (98%), acetic acid (96%), potassium chloride, potassium iodide, potassium bromide, iodine, fast blue salt B, Uvasol methanol-d_4, tetramethylsilane, trifluoroacetic acid, hydrogen peroxide (3%) solution stabilized, and natural product reagent A were from Roth (Karlsruhe, Germany). Silica gel 60 for column chromatography, 20 cm × 20 cm aluminium-backed silica gel $60F_{254}$ HPTLC (high-performance thin-layer chromatography) plates for nano-TLC (thin-layer chromatography), 10 cm × 10 cm HPTLC plates coated with a 0·2 mm layer of RP_{18} WF_{254}, L(+)-ascorbic acid, Merckoquant peroxide analytical test strips, and Titrisol sodium thiosulphate solution (0·01 N) were all p.a. quality, unless stated otherwise, from Merck (Darmstadt, Germany). Maize starch was from Roquette (Lille, France). Helium 4·6 and nitrogen class 4 were from Messer Gries- heim (Griesheim, Germany). Cellulose acetate filters, 0·45 μm, were obtained from Sartorius (Göttingen, Germany).

Isolation of hyperforin

Drug. Blossoms of *Hypericum perforatum* L. were collected in July 1996 on the Schwäbische Alb (Roßberg) and verified by characteristic botanical description (Roth 1990). The blossoms were stored at −20°C.

Preparation of the crude extract. Blossoms (4·6 g) were extracted three times for 5 min each with *n*-hexane (30 mL), containing 0·01% (m/m) saturated ascorbic acid solution, by means of an Ultra Turrax type TP 18/10 N (Janke & Kunkel, Staufen, Germany). The fractions were combined and concentrated to 2 mL by rotary evaporation (Büchi, Goeppingen, Germany).

Silica gel chromatography. Lipophilic substances were separated on a 23 cm × 1·25 cm i.d. silica gel

column with 33 : 35 : 30 (v/v) *n*-hexane–acetone–*t*-butyl methyl ether containing 0·1% (m/m) ascorbic acid as mobile phase; the flow rate was 1·2 mL min^{-1}. Fractions (10 mL) were collected and the appropriate fractions (as monitored by HPTLC) were combined, evaporated and concentrated to 2 mL.

HPTLC. Fractions (10 μL) containing hyperforin were identified by HPTLC on silica gel 60 F_{254} with *n*-heptane–acetone–*t*-butyl methyl ether-96% acetic acid, 33 : 35 : 30 : 2 (v/v), as mobile phase. Sample detection was by UV (ultraviolet) absorbance at 254 nm and detection of hyperforin was enhanced by spraying with fast blue salt B 0·5% in water (yellow colour). The retention factor (R_F) of hyperforin was 0·45. Residue analysis for substances adsorbed completely on the preparative RP_{18} material was performed with RP_{18} plates and acetonitrile–twice distilled water, 89·5 : 10·5 (v/v), as mobile phase. The R_F of hyperforin was 0·2.

Preparative HPLC. Concentrated fractions from silica gel chromatography (200 μL) were purified by preparative HPLC on a 25 cm × 10 mm i.d., 7-μm VarioPrep 100 C_{18} column (Macherey-Nagel, Düren, Germany) combined with a 40 mm × 10 mm i.d., 20-μm Nucleoprep 100 C_{18} guard column. The mobile phase, helium-sparged acetonitrile–twice-distilled water, 89·5 : 10·5 (v/v) was supplied at a flow rate of 6·2 mL min^{-1} by means of an LCP 4000 pump (Biotek, Sulzbach, Germany). Detection at 272 nm was achieved with an LCD 2082 UV-detector (Biotek). The analysis was supported by Class-CR 10 software, version 1·3 (Shimadzu, Düsseldorf, Germany). The hyperforin peak was cut from the flow between 16·4 and 17·8 min. The azeotrope phase was removed by rotary evaporation.

Freeze-drying. The remaining hyperforin–water emulsion was transferred to vials (2·0 mL), frozen at −30°C and freeze-dried by means of a Lyovac GT 2 freeze dryer (Finn-Aqua, Hürth, Germany) equipped with a Trivac D8 B vacuum pump (Leybold, Köln, Germany).

Storage conditions. The vials were stored at −196°C (reference sample) and −30°C. Samples were also stored at −20, +4 and +20°C both under nitrogen and in a normal atmosphere. Stability testing was performed by analytical HPLC after 1 day and 1, 2, 5, 20 and 32 weeks as described below.

Analysis

HPTLC. The purity of hyperforin was double–checked by HPTLC on silica gel with *n*-heptane–acetone–*t*-butyl methyl ether–96% acetic acid, 33 : 35 : 30 : 2 (v/v) and toluene-formic acid ethyl ester-formic acid, 5 : 4 : 1 (v/v) as mobile phases. The R_F values were 0·45 and 0·8 respectively. UV-detection was performed at 254 nm (fluorescence quenching); detection sensitivity was enhanced by spraying with fast blue salt B 0·5% in water (yellow colour).

Analytical HPLC. The stored samples were dissolved in methanol (2·0 mL) and analysed by HPLC on a 25 cm × 4 mm i.d., 5 μm ET 100 C_{18} column (Macherey-Nagel) with acetonitrile–twice-distilled water, 89·5 : 10·5 (v/v), as mobile phase delivered at a flow rate of 1·2 mL min^{-1} by means of a LC-6A pump (Shimadzu) with a 20-μL Rheodyne injection loop. Hyperforin was detected by means of a SPD-6AV spectrophotometer (Shimadzu) operating at 272 nm. Software was Class CR-10, version 1.2 (Shimadzu). Under these conditions the retention time of hyperforin was 8·6 min, i.e. the k value was 2·7. Calibration was performed with pure hyperforin as reference standard. The calibration data are presented in Table 1.

Diode-array detection. Pure hyperforin (0·5 mg) was dissolved in 0·5 mL methanol. The column, flow rate and loop were as described for analytical

Table 1. Calibration results from analytical HPLC of hyperforin*.

Model	y(x)	= b1(x − E[xc]) + E[yc]
Number of calibration samples	nc	48
Slope, or sensitivity	b1	7·4548E + 05 AUC mg^{-1}
Calibration function	y(x)	= 745480·774 [x − 0·343] + 255993·897
Limit of detection	xdtc	0·00533 mg mL^{-1}
Limit of quantitation	xdet	0·01715 mg mL^{-1}
Correlation coefficient	r	0·9997

*According to Ebel (1992).

HPLC. Detection was performed with a 1040A diode array detector (DAD; Hewlett-Packard, Böblingen, Germany) operating between 190 and 600 nm. Three DAD spectra, recorded on both flanks of the hyperforin peak and at its maximum, were compared. No differences between the three spectra were found; this was expressed as a match factor of 1000.

NMR. ^{1}H NMR (nuclear magnetic resonance) spectroscopy experiments (Table 2) were performed on 2-mg mL^{-1} solutions of hyperforin in methanol. 400 MHz spectra were acquired on a Bruker (Karlsruhe, Germany) WM 400 instrument. Data collection and processing were performed with Aspect 2000 software (Bruker).

FTIR. FTIR (Fourier-transformed infrared) spectra (Table 3) as potassium bromide discs were recorded between 4000 and 400^{-1} cm on an IFS 48 spectrometer with an Aspekt 1000 data system (both Bruker).

LC-(+)ESI-MS-MS. Samples containing pure hyperforin were analysed by use of an HPLC system linked to an API III Taga 6000 E triple-quadrupole mass spectrometer equipped with an electrospray-ionization source (LC-(+)ESI-MS-MS; Perkin-Elmer-Sciex, Foster City, USA). Full scans were recorded over the mass range 200–1000 amu. Samples (10 μL) were injected on to a 250 mm × 2 mm i.d., 5-μm Hypersil ODS C_{18} column (Grom, Herrenberg, Germany) and eluted with acetonitrile–twice-distilled water–trifluoroacetic acid, 89·5 : 10·4 : 0·1 (v/v) at 0·2 mL min^{-1} by means of an Applied Biosystems (Foster City, USA) ABI 140A pump combined with a Linear (Reno, USA) 204 UV/VIS-detector operating at 272 nm with a post column split of 1 : 20. All data were processed by Tune version 2·5 and MacSpec version 3.3 software.

Table 2. ^{1}H NMR data for hyperforin.*

H	δ (ppm)	(m)†	J (Hz)‡
H_{12}	1·04	d	6
H_{13}	1·2	d	6
H_{24}	1·52	s	–
H_{16}	1·88	m	–
H_{21}	2·02	m	–
H_{22}	4·87	t sept	7·5/1·4
H_{27}	4·96	t sept	5/1·4
H_{17}	5·02	t sept	7·5/1·4
H_{32}	5·15	t sept	7·5/1·4

*Classification of H atoms according to Ruecker et al (1995); (deuteromethanol, 400 MHz). †s, singlet; d, doublet; t triplet; m, multiplet; s septulet ‡Other hydrogen atoms could not be identified.

Table 3. FTIR data for hyperforin.*

Wavelength (cm^{-1})	Intensity†	Structure
3329	m	OH
2971	s	CH_3
2925	ss	CH_2
1732	s	C=O
1676	m	C = C
1456	ss	CH_2, CH_3
1237	m	C–C
830	w	C=C

*KBr disk. †w, weak; m, medium; s, strong; ss, very strong.

Peroxide detection. Acetic acid (96%)–chloroform (2 : 3, v/v; 30 mL) was placed in a 200-mL round-bottomed flask and heated to boiling. After addition of saturated potassium iodide–iodine solution (0·5 mL) the sample (2 mL) was added. After 3·0 min water (100 mL) and preheated maize starch solution (20%, m/v; 5·0 mL) was added and the sample was titrated with 0·01 N Titrisol sodium thiosulphate solution. The peroxide value (POV) was calculated by use of equation 1.

$$POV = 10 \times (n_1 - n_2)/w \qquad (1)$$

where n_1 is the titration volume (mL), n_2 the blind volume (mL), and w the amount of sample (mL). The blind value should not exceed 0·1 mL of 0·01 N sodium thiosulphate solution (Maier 1990). Positive testing was performed by use of 0·03 mL 3% (m/m) hydrogen peroxide and a second check of peroxide value was performed by use of Merckoquant analytical peroxide test-strips.

Statistical analysis

Means and 95% confidence intervals were calculated from results of analysis of the stability of hyperforin over a period of 8 months. The data were also checked by means of the multiple-range test (Sachs 1997) designed for comparing more than one group of results with a control.

Results and Discussion

The extraction efficiencies of different solvents for hyperforin are given in Table 4. Better results were obtained with petroleum ether than with methanol, *n*-hexane and *n*-heptane. Because of the lower boiling point and therefore better evaporation behaviour, *n*-hexane was chosen for the extraction despite its lower extraction yield and greater

toxicity. Because of rapid degradation in lipophilic organic solvents, turbo extraction was performed with an Ultra Turrax at 20 000 rev min^{-1}. Hyperforin was exhaustively extracted from the dried herb and pre-purification of the extract on a silica gel column minimized the amount of substances with high affinity for the RP_{18} phase. To achieve reproducible preparative HPLC over a long period of time, a self-packed guard column was used to saturate the mobile phase and remove any remaining extremely lipophilic substances. For consistent protection against oxidation it was essential that ascorbic acid was added to both the extraction solvent and to the mobile phase used for column chromatography and that the HPLC mobile phase was sparged with helium. If these procedures were not followed the amounts of peroxides present increased after each step of the isolation process (Table 5). Freeze-drying was performed under nitrogen. The amount of hyperforin obtained was 5 mg from extraction of 4·6 g herbal drug; this is equivalent to a total of 3% hyperforin in the dried herb, which means that the total loss during the isolation procedure was 85%. This is because of the sensitivity of hyperforin to oxidation in the apolar organic solvents used for concentration during isolation, sensitivity to light, and losses during chromatography.

Table 4. Extraction efficiencies of various solvents for hyperforin compared with petroleum ether (100%).

Solvent	Extraction efficiency (%)	Boiling point (°C)
Methanol	92·5	65
n-Hexane	94	68·9
n-Heptane	97	98·4
Petroleum ether	100	70–80

Because of the temperature sensitivity of hyperforin, *n*-hexane was chosen for further investigations despite its lower extraction efficiency compared with *n*-heptane and petroleum ether.

Table 5. Comparison of peroxide levels with and without addition of ascorbic acid during the isolation of hyperforin.

Isolation step	Amount of peroxide ($mg L^{-1}$)
n-Hexane	0
Extract	6
Concentrated extract	25
Concentrated extract + ascorbic acid	0
After column chromatography	23
After column chromatography + ascorbic acid	1
Concentrated column chromatography extract	13
Concentrated column chromatography extract + ascorbic acid	0
Preparative HPLC	2
Preparative HPLC + helium	0

The most critical step is the concentration of the crude extract. Prevention of oxidation eliminates peroxide formation and enrichment.

Compound identity was confirmed by ^1H NMR, MS and FTIR spectroscopy and comparison with literature data (Gurevich et al 1971; Bystrov et al 1978; Maisenbacher 1991) (Tables 2 and 3). Essential purity monitoring of the isolate for trace impurities and degradation products was also performed. Thin-layer chromatography is still necessary for basic 100% control, because even substances with zero R_F values can be detected, in contrast with HPLC techniques. Impurities in the hyperforin could not be traced either with the two normal-phase HPTLC methods or by reversed chromatography on RP_{18}. The 190 to 600 nm diode-array spectrum also confirmed the absence of impurities—the match factor of the hyperforin peak was 1000, i.e. similar spectra were recorded on both flanks of the peak and at its maximum. Quantification of low-level impurities often poses a difficult analytical problem for HPLC if conventional detectors are used, because such impurities are often very similar to the parent compound both in spectral properties and chromatographic mobility. An LC-(+)ESI-MS-MS technique was, therefore, chosen to achieve higher sensitivity and selectivity. A full scan spectrum of hyperforin (Figure 2) shows three main peaks at m/z 537, 559 and 575. That at m/z 537 is of the protonated molecular ion of hyperforin $(m/z+1)^+$; the peaks at m/z 559 and 575 arise from the Na^+ (+22 amu) and K^+ (+39 amu) adducts. This clustering is very common with electrospray ionization. The peaks below 537 are all fragments of hyperforin, as is apparent from the daughter-ion scans of $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ (Figure 2) because each mass peak in the total spectrum can be detected in one of the three daughter ion scans. Comparison of the total-ion current (TIC) chromatogram obtained from mass spectrometry with the chromatogram recorded during online UV-detection at 272 nm revealed no evidence of the presence of impurities (Figure 3), and so the possibility of the presence of impurities lacking an UV-detectable chromophore could be discounted.

Stability testing over 8 months storage (Table 6) indicated no statistically significant difference (95% confidence level) between storage at −196, −30 and −20°C under nitrogen. Storage at −20°C and above resulted in a significant decline in hyperforin content. Under all the conditions tested the hyperforin content declined less if stored under

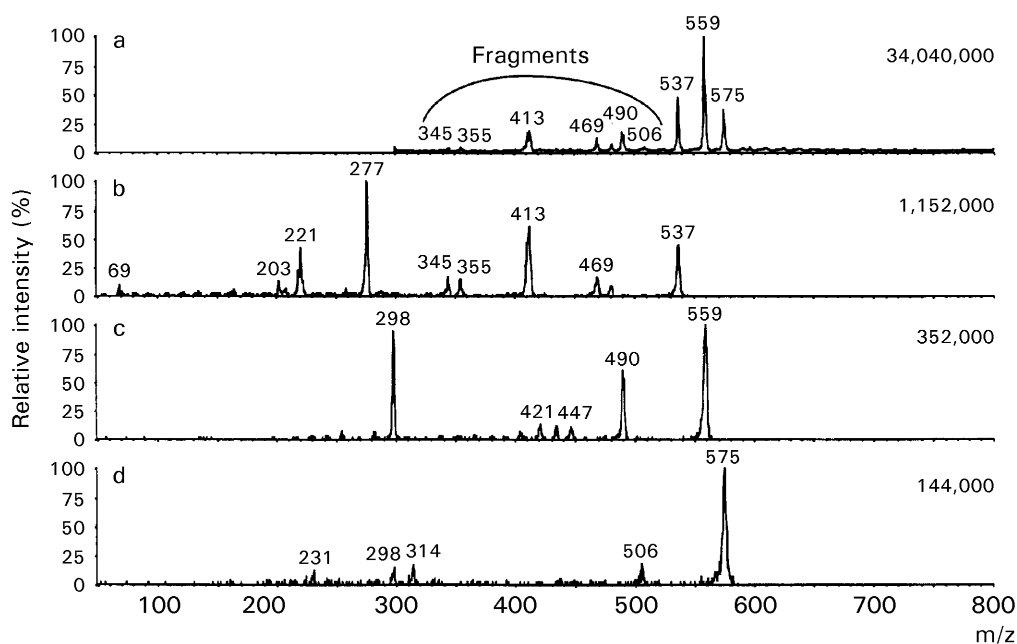

Figure 2. Positive-ion electrospray-ionization tandem mass spectrometry of hyperforin: full scan (a) and daughter-ion scans of $[M+H]^+$ (b), $[M+Na]^+$ (c), and $[M+K]^+$ (d). Mass 537 in the full scan corresponds to hyperforin in the positive-ion mode (m/z +1). The increase in mass to 559 and 575 is attributed to formation of the Na^+ (+22 a.m.u.) and K^+ (+39 a.m.u.) adducts, respectively. The peaks below 537 are all fragments of hyperforin, because each mass peak in the total spectrum can be detected in one of the three daughter-ion scans.

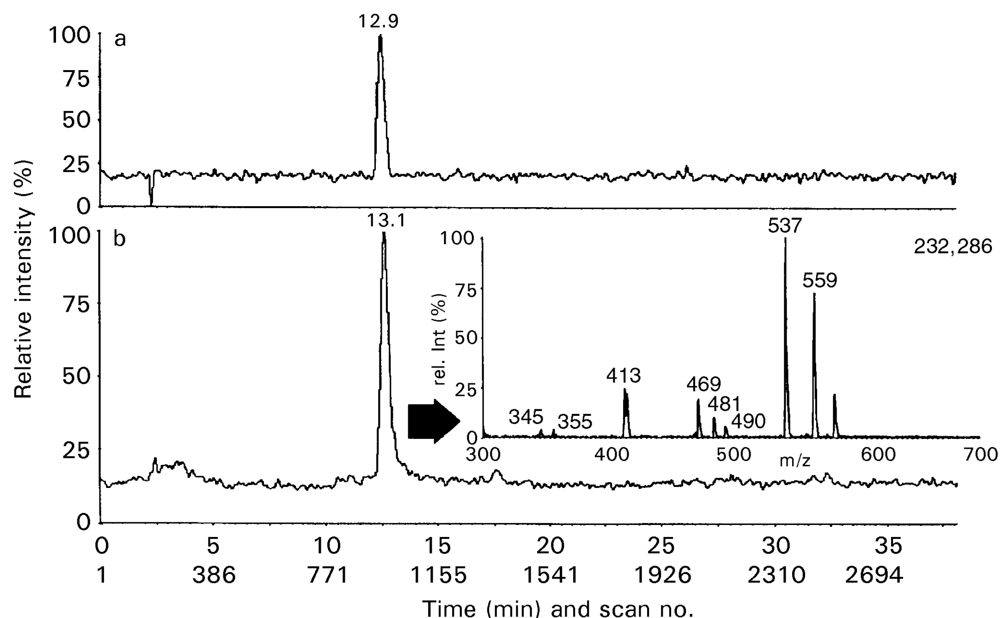

Figure 3. Comparison of the total ion-current (TIC) chromatogram of hyperforin (below) with the LC-UV chromatogram (above) recorded in parallel. No impurities were detected by use of the full-scan mode of MS detection. This eliminates the possibility of the presence of impurities with no UV-detectable chromophore.

nitrogen. In contrast with the results obtained for isolated hyperforin, the hyperforin content of the dried herb and of preparations containing extracts of *Hypericum perforatum* L. was indicative of better stability. This could be attributed to the presence of antioxidative substances in the matrix of the plant cell.

In conclusion, this study provides evidence that hyperforin can be isolated by preparative HPLC from frozen flower heads of *Hypericum perforatum*

Table 6. Multiple range test showing mean values of hyperforin content after 8 months storage under different conditions.

Test conditions (°C)	Sample size	Mean (AUC)	Homogeneous groups
−196	16	$1{\cdot}543 \times 10^6$	x
−20 + nitrogen	15	$1{\cdot}500 \times 10^6$	xx
−30	15	$1{\cdot}427 \times 10^6$	xxx
−20	15	$1{\cdot}348 \times 10^6$	xx
4 + nitrogen	15	$1{\cdot}308 \times 10^6$	xx
4	15	$1{\cdot}174 \times 10^6$	xx
20 + nitrogen	15	$1{\cdot}138 \times 10^6$	x
20	15	$0{\cdot}846 \times 10^6$	x

The vertical columns of 'x's show those samples that are not significantly different (homogeneous groups) at the 95% confidence interval LSD (least significant differences). The value obtained after storage at −196°C was set to 100%. Because of the sensitivity to oxidation the hyperforin content under each set of test conditions declines less if nitrogen is used. There was no statistically significant difference between results obtained after storage at −20°C under nitrogen or at −30°C under a normal atmosphere and those obtained after storage at −196°C. Nevertheless, it is recommended that long-term storage of hyperforin is at −70°C under nitrogen.

L. with a purity of 99·9% as determined by HPLC (AUC, 190–600 nm DAD and UV 272 nm), HPTLC and LC-ESI(+)-MS-MS. Identity was confirmed by FTIR and ^{1}H NMR spectroscopy and by MS. Exclusion of light and consistent protection from oxidation by use of L-(+)-ascorbic acid, nitrogen and helium proved essential during the isolation process. This treatment removed peroxides present at the start of the extraction process and prevented the formation of more peroxides during the isolation procedure. Acceptable shelf-life of the isolated hyperforin, compared with that achieved by storage under liquid nitrogen (−196°C), was achieved only by storage at −20°C under nitrogen or at −30°C under a normal atmosphere. Nevertheless, because of the degradation of the pure hyperforin long-term storage should be at −70°C under nitrogen. A further communication will deal with technological possibilities of stabilizing hyperforin at higher temperatures.

References

Bladt, S., Wagner, H. (1994) Inhibition of MAO by fractions and constituents of Hypericum extract. J. Geriatr. Psych. Neurol. 7 (Suppl. 1): 273–275

Bombardelli, E., Morazzoni, P. (1995) *Hypericum perforatum*. Fitoterapia 66: 43–68

Butterweck, V., Wall, A., Liefländer-Wulf, U., Winterhoff, H., Nahrstedt, A. (1997) Effects of the total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity. Pharmacopsychiatry 30 (Suppl. 2): 117–124

Bystrov, N. S., Dobrynin, V. N., Kolosov, M. N., Popravko, S. A., Chernov, B. K. (1978) Chemistry of hyperforin. IX Structure of hyperforin. Bioorg. Khim. 4: 791–797

Chatterjee, S. S., Noeldner, M., Koch, E., Erdelmeier, C. (1998) Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility. Pharmacopsychiatry 31 (Suppl. 1): 7–15

Commission E of the German Federal Public Health Department (1984, correction 1989) Monograph Hyperici herba, Bundesgesundheitsamt, Berlin

Ebel, S. (1992) Würzburger Skripten zur Analytik, Reihe Statistik, 4th edn, Institute of Pharmacy and Chemistry, University of Würzburg, pp 43–88

Erdelmeier, C. A. J. (1998) Hyperforin, possibly the major non-nitrogenous secondary metabolite of *Hypericum perforatum* L. Pharmacopsychiatry 31 (Suppl. 1): 2–6

Gurevich, A. I., Dobrynin, V. N., Kolosov, M. N., Popravko, S. A., Ryabova, I. D., Chernov, B. K., Derbentseva, N. A., Aizenman, B. E., Gargulya, A. D. (1971) Hyperforin, an antibiotic from *Hypericum perforatum* L. Antibiotiki 16: 510–513

Laakmann, G., Schuele, C., Baghai, T., Kieser, M. (1998) St John's wort in mild to moderate depression: the relevance of hyperforin for the clinical efficacy. Pharmacopsychiatry 31 (Suppl. 1): 54–59

Linde, K., Ramirez, G., Mulrow, C. D., Pauls, A., Weidenhammer, W., Melchart, D. (1996) St John's wort for depression—an overview and meta-analysis of randomised clinical trials. Br. Med. J. 313: 253–258

Lohse, M. J., Mueller-Oerlinghausen, B. (1997) Psychopharmaka. In: Schwabe, U. (ed.) Arzneiverordnungsreport '97, Gustav Fischer, Stuttgart, pp 470–474

Maier, H. G. (1990) Peroxidzahl. In: Maier, H. G., Lebensmittel und Umweltanalytik, 1st edn, Steinkopff, Darmstadt, pp 44–45

Maisenbacher, P. (1991) Untersuchung zur Analytik von Johanniskrautöl. Thesis, University of D-Tübingen

Mueller, W. E., Singer, A., Wonnemann, M., Hafner, U., Rolli, M., Schäfer, C. (1998) Hyperforin represents the neurotransmitter re-uptake inhibiting constituent of Hypericum extract. Pharmacopsychiatry 31 (Suppl. 1): 16–21

Ostrowski, E. (1988) Untersuchungen zur Analytik, ^{14}C-Markierung und Pharmakokinetik phenolischer Inhaltsstoffe von *Hypericum perforatum* L. Thesis, University of D-Marburg

Roth, L. (1990) Botanik von Hypericum. In: Roth, L. (ed.) Hypericum-Hypericin: Botanik, Inhaltsstoffe, Wirkung, 1st edn, Ecomed, Landsberg, pp 15–87

Ruecker, G., Manns, D., Hartmann, U., Bonsels, U. (1995) A C_{50}-hydroperoxide from *Hypericum perforatum* L. Arch. Pharm. 328: 725–730

Sachs, L. (1997) Applied Statistics, 8th edn, Springer, Berlin, pp 393–400

Schellenberg, R., Sauer, S., Dimpfel, W. (1998) Pharmacodynamic effects of two different Hypericum extracts in healthy volunteers measured by quantitative EEG. Pharmacopsychiatry 31 (Suppl. 1): 44–53

Sparenberg, B., Demisch, L., Hölzl, J. (1993) Untersuchungen über antidepressive Wirkstoffe von Johanniskraut. Pharm. Ztg. Wiss. 138: 50–54

Suzuki, O., Katsumata, Y., Oya, M., Bladt, S., Wagner, H. (1984) Planta Med. 50: 205–284

Wichtl, M. (1997) Hyperici herba. In: Wichtl, M. (ed.) Teedrogen und Phytopharmaka, 3rd edn, WVGes, Stuttgart, pp 309–312