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Quantitative analysis of the major constituents of St John's wort with HPLC-ESI-MS

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Abstract

A method was developed to profile the major constituents of St John's wort extracts using high-performance liquid chromatography–electrospray mass spectrometry (HPLC-ESI-MS). The objective was to simultaneously separate, identify and quantify hyperforin, hypericin, pseudohypericin, rutin, hyperoside, isoquercetrin, quercitrin and chlorogenic acid using HPLC-MS. Quantification was performed using an external standardisation method with reference standards. The method consisted of two protocols: one for the analysis of flavonoids and glycosides and the other for the analysis of the more lipophilic hypericins and hyperforin. Both protocols used a reverse phase Luna phenyl hexyl column. The separation of the flavonoids and glycosides was achieved within 35 min and that of the hypericins and hyperforin within 9 min. The linear response range in ESI-MS was established for each compound and all had linear regression coefficient values greater than 0.97. Both protocols proved to be very specific for the constituents analysed. MS analysis showed no other signals within the analyte peaks. The method was robust and applicable to alcoholic tinctures, tablet/capsule extracts in various solvents and herb extracts. The method was applied to evaluate the phytopharmaceutical quality of St John's wort preparations available in the UK in order to test the method and investigate if they contain at least the main constituents and at what concentrations.

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Introduction

During the past 15 to 20 years preparations containing St John's wort (*Hypericum perforatum* L.) extracts have gained popularity as antidepressants. They are produced in a variety of ways, using different parts of the plant, and are from different geographical sources. The main active constituents of *Hypericum* extracts are relatively well known and include hyperforin, the naphthodianthrones hypericin and pseudohypericin, 13, II 8-biapigenin, amentaflavone, rutin, hyperoside, isoquercetrin, quercitrin, quercetin and chlorogenic acid (Barnes et al 2001; see Figure 1). Some preparations contain only dried plant material whereas others contain standardised extracts, often standardised to the content of both hyperforin and hypericin.

Various methods have been published for the analysis of *Hypericum* extracts. The European Pharmacopoeia monograph for *Hyperici* herba uses a visible (VIS) spectroscopic method for the assay of total hypericins with absorption measurements at 590 nm. This does not allow for a selective measurement of hypericin and pseudohypericin since other compounds that absorb at this wavelength, such as chlorophyll, will result in an overestimation of the content of hypericin and its derivatives. Also, it only assays for the naphthodianthrone content and therefore cannot be used for the quantification of the other compounds. Fluorimetric methods have also been used and are more selective and sensitive than VIS spectroscopy, although overestimation of hypericins is still possible (Klein-Bischoff & Klumpp 1993).They will not be useful for the quantification of hyperforin-type compounds as these are not fluorescent. Most other previously published methods have used HPLC coupled to UV/VIS and/or fluorimetric detection (Liu et al 1992; Kartnig et al 1996; Repcak and Martonfi 1997; Gerlie et al 2001; Tolonen et al 2003). Some methods additionally use MS and tandem

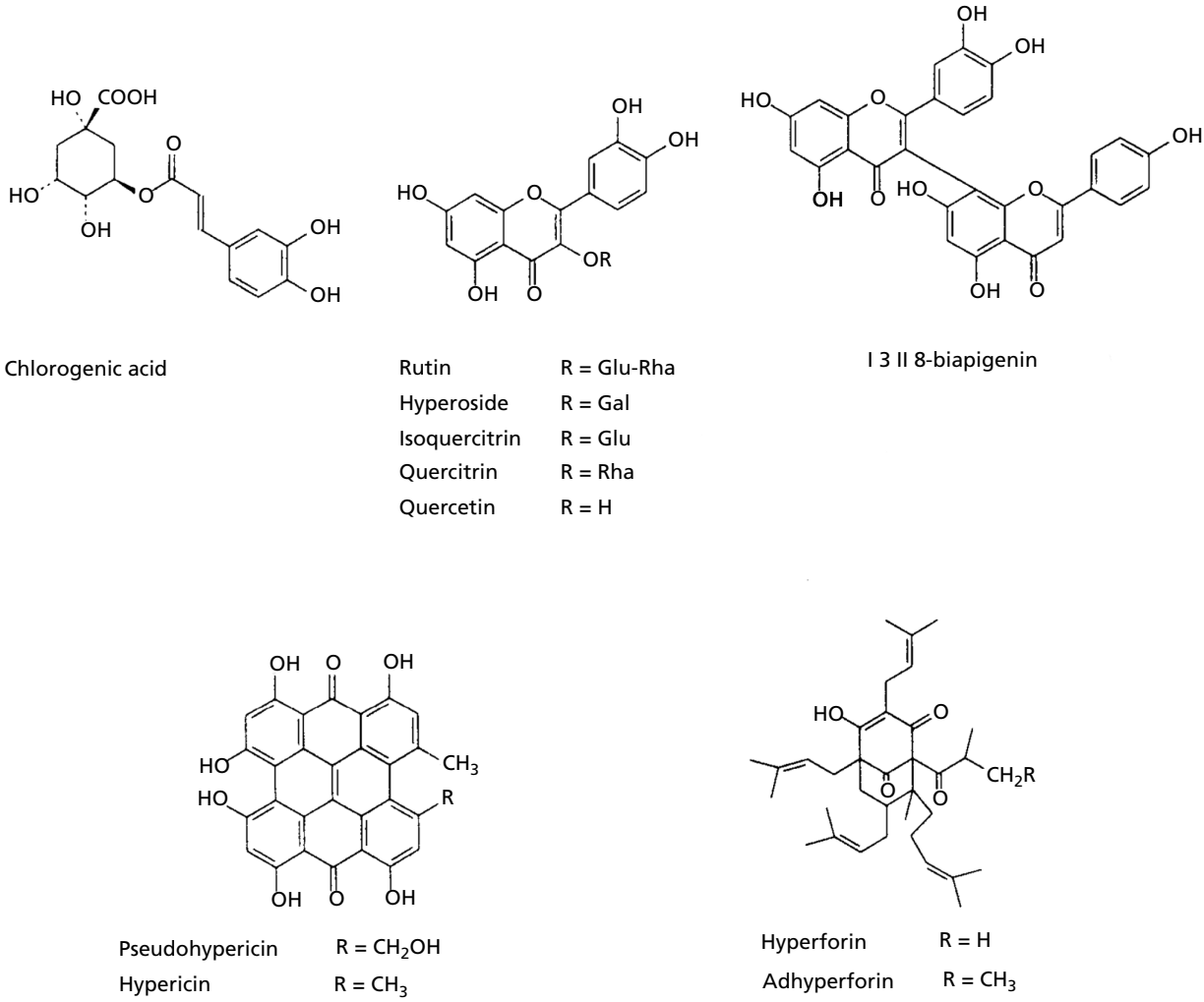

Figure 1 The chemical structures of the main active constituents of St John's wort.

mass spectrometric (MS-MS) detection (Piperopoulos et al 1997; Brolis et al 1998; Hansen et al 1999; Lay 2000; Liu et al 2000; Mauri & Pietta 2001; Huck et al 2002; Pirker et al 2002; Tolonen et al 2002).

Most HPLC methods make use of reverse-phase chromatography, usually with the most commonly used C-18 packing. Generally a good selectivity and good resolution for closely related compounds such as hypericin and pseudohypericin, and hyperforin and adhyperforin have been reported. These systems can, however, result in long retention times for the more lipophilic compounds. In this study, a phenyl hexyl column was used to reduce retention times. The analysis of hypericins can pose several problems, most of which were encountered during attempts to reproduce some published methods and subsequent method development. These include broad tailing peaks and solubility issues. Our attempts to reproduce several of the published methods produced broad indistinct peaks for hypericins with varying retention times. The analysis was therefore split into two protocols to obtain an accurate and reproducible quantification of hypericins: one for the more polar flavonoids and glycosides and the other for hypericins and hyperforin.

Most of the published methods with MS detection have used it for identification and confirmation purposes only. Quantification is usually with other methods. Only two methods have used MS for quantification: one reported the quantification of hypericins and hyperforin (Tolonen et al 2002), whereas the other reported the quantification of hypericins only (Mauri & Pietta 2001). The method described here uses HPLC-ESI-MS for the simultaneous identification and quantification of all the main constituents, which is simpler than combining two detection methods. HPLC-MS, especially selected ion recording where the mass spectrometer is set to detect only the required ions, is more specific and sensitive than HPLC-UV detection and can provide certainty where there are co-eluting peaks, which is possible with *Hypericum* extracts. It also allows the elucidation of peaks not identified by the comparison of retention times with standard reference compounds.

The method developed was applied to the investigation of a random selection of extract-based St John's wort

preparations in order that the products could be compared with respect to their phytochemical composition. The claims made by the manufacturers as to the content of any of these constituents in these preparations may not be reliable because of their general instability and the possibility of decay. For example, a study in the USA of eight products found that for several products the actual content of hypericins ranged from 57 to 130% of the labelled amount (de los Reyes & Koda 2002). A high variability in the content of hyperforin was also found. Variability in the content of flavonoids as well as hypericins and hyperforin has also been reported (Bergonzi et al 2001). All constituents could be accurately and reproducibly quantified from capsules, tablets and tinctures as well as dry herbal material.

This method can be used to study the constituents of *Hypericum* extracts with MS or tandem mass spectrometry (MS-MS); it was used subsequently to investigate the rate and products of degradation of hypericins and hyperforin in the finished product, dry extract and the herb by coupling to MS-MS without further adaptation.

Materials and Methods

Chemicals and instrumentation

Hypericin and chlorogenic acid (98%) were obtained from Fluka Chemicals (Dorset, UK) (95% HPLC). Pseudohypericin (98% HPLC) was obtained from Calbiochem (CA). Hyperforin (sodium salt, 58% pure) was kindly donated by Lichtwer Pharma AG (Berlin). Rutin (99%) was obtained from Avocado Research UK, hyperoside from Biochem UK, isoquercitrin from Extrasynthese (Genay, France) and quercetin (97%) and quercitrin (85%) from Sigma Chemicals (Dorset, UK). Acetonitrile, methanol and acetone were HPLC grade and obtained from Sigma Chemicals (Dorset, UK). Water was purified with a UsF Elga system. Formic acid (97%) was obtained from Sigma Chemicals (Dorset, UK).

HPLC separations were carried out with a Waters Alliance 2690 Separations Module. MS detection was with a Finnigan Navigator quadrupole benchtop mass spectrometer. The column used was a Luna phenyl hexyl column (150×4.6 mm, 5 μm particle size) fitted with a guard column of the same material. The samples were filtered prior to analysis with Millipore Millex-GS filter units of 0.22 μm pore size. An Erga column oven was used to regulate the temperature of analysis at 30°C. The commercial St John's wort preparations analysed are listed in Table 1. The Kira St John's wort products were kindly donated by Lichtwer Pharma UK and the others were purchased from pharmacies and health food stores. All had ≥8 months to the expiry date.

Table 1 The commercial St John's wort preparations analysed in this study

Product	Labelled content of hypericin (μg)
Kira St John's wort tablets (s)	900
Kira St John's wort tablets (u)	300
Boots St John's wort tablets (u)	900
Bioforce St John's wort tablets (u)	330
Healthilife St John's wort capsules (u)	180
Gerrard House St John's wort tablets (u)	–
Good 'n' Natural St John's wort capsules (s)	450
Herb Tech St John's wort capsules (s)	900
Health Aid St John's wort tablets (s)	900
Ultimate Nutrition St John's wort capsules (s)	1050
Hofels St John's wort capsules (s)	900

s, standardised product; u, unstandardised product.

HPLC-ESP-MS quantification

The separation method consisted of two protocols: Protocol 1 for glycosides and flavonoids and Protocol 2 for hypericins and hyperforin. They were developed as a result of preliminary work. Protocol 1 is a substantial modification of the method described by Kartnig et al (1996) while Protocol 2 is a new method.

Protocol 1

The following gradient was set up with solvent A (acetonitrile) and solvent B (water) with 0.5% formic acid: between 0 and 27 min, 16% A; from 27 to 30 min, a linear change from 16 to 32% A; from 30 to 35 min, 32% A; from 35 to 40 min, a linear change from 32 to 16% A. The flow rate was 1 mL min^{-1}. An injection volume of 10 μL was used.

Protocol 2

The analysis of hypericins and hyperforin was achieved with an isocratic elution using a mobile phase of acetonitrile: water:formic acid:methanol (70:4.95:0.05:25). The flow rate was maintained at 1.2 mL min^{-1}. An injection volume of 10 μL was used. The column was washed with isopropyl alcohol after carrying out each set of experiments in order to elute any remaining lipophilic compounds, such as chlorophylls.

ESI-MS detection

The MS data were recorded as total ion chromatograms (TIC), produced by summing the ion currents for all masses in the individual mass spectra acquired each second, and selective ion monitoring (SIM), in which the instrument is set to record only the ion currents from selected masses, simultaneously. Quantification was achieved with SIM as this is more specific and sensitive than TIC.

The ionisation parameters were optimised by varying the cone voltage, the source heater temperature, the nebulising gas flow, the potential of the electrospray capillary and the flow rate of the mobile phase into the mass spectrometer. The cone voltage values evaluated were 20, 25 and 30 V. Drying gas flows of 350, 400 and 430 L h^{-1} were evaluated. The source heater temperature levels tested were 150, 180 and 200°C. The potential of the electrospray capillary was varied, with 3.0, 3.2 and 3.5 kV evaluated. For Protocol 1 the ESI values used were capil-

lary voltage 3.5 kV, HV lens 0.5 kV, cone voltage 30 V, skimmer 1.5 V, skimmer offset 5 V, RF lens 0.1 V and source heater maintained at 180°C. Nitrogen was used as the nebulising gas at a flow rate of 430 L h^{-1}. The analysis was performed using ESI in the negative ionisation mode. The flow from the HPLC into the MS inlet was maintained at 0.2 mL min^{-1} to achieve the best sensitivity.

For Protocol 2 the cone voltage was increased to 40 V and the source heater temperature decreased to 150°C. A nebulising gas flow of 400 L h^{-1} was used. The scan range used for both protocols was 100–1000 amu.

External standardisation method

Quantification was performed by preparing calibration curves for all nine standards using six calibration points, after establishing their linear response range in ESI-MS. The linear response range is the concentration range within which the response varies proportionately with the concentration. This was achieved by analysing a range of concentration levels for each standard and by subsequent visual inspection of the plot of response as a function of concentration and statistical analysis of the data (method of least squares). Six standard concentration levels were prepared by serial dilution of a stock solution in methanol. For Protocol 1, a mixture of standards of known concentration for each compound was prepared in one vial, with a total of six vials as six calibration points used for each standard. Thus only one set of analyses was required for all six compounds for each calibration point, with quantification being performed separately by the data system subsequent to the analysis. For Protocol 2, calibration curves were prepared for all three analytes using six concentration levels. Calibration curves were established each time an analysis was performed to account for any possible variation in ionisation due to the condition of the instrument. All standards were analysed in triplicate, with blanks in between, immediately before the analysis of the samples. All samples were kept in the dark and stored at −20°C under nitrogen when not in use. Quantification was achieved with Mass Lab Quantify software.

Specificity of the method

The specificity of the method for the analytes was checked by comparison with standards. A mixture of standards was analysed by adding one standard at a time to check for interferences and the resulting chromatogram was compared to sample chromatograms. Comparison of retention times and MS spectra were used as identification tests. Blank chromatograms were obtained by injecting methanol and checked for any interfering peaks using selective ion monitoring. MS was used to check analyte peak purity.

Limit of detection and limit of quantification

Calibration curves using a low concentration range were generated for each analyte to establish the limit of detection (LOD), i.e. the lowest amount detectable with a 3:1 signal-to-background ratio. The limit of quantification (LOQ) was also established using a signal-to-background ratio approach (10:1) and was the lowest amount quantifiable with accuracy and precision of less than 20%. The analysis was performed in triplicate. The standard compounds were dissolved in methanol.

Accuracy, precision and repeatability of analysis

Accuracy (based on three concentration levels and three replicates), precision (intra-assay variability based on three concentration levels and three replicates) and repeatability (inter-day variability based on six concentration levels and three replicates) were evaluated for the quantification of each constituent.

Product analysis

The products were extracted and analysed in duplicate. Each sample was subjected to three HPLC analyses.

Extraction method

One tablet was weighed and crushed. The contents of a capsule were emptied out and used directly. The capsule was weighed before and after emptying. Two hundred milligrams (± 10%) of the powder from the tablet/capsule was then weighed accurately and placed in a 25-mL amber volumetric flask. Twenty millilitres of methanol was added and the flask sonicated for 1 h. The extract was filtered using filter paper into a 50-mL amber volumetric flask and the residue re-extracted in 20 mL of methanol in the original 25-mL flask for another hour. The supernatent of the second extraction was added to the 50-mL flask at room temperature. The flask was then made up to volume, and the extract filtered and analysed.

Results and Discussion

Method development

Method development posed several analytical problems, mainly due to the poor solubility of hypericin and pseudohypericin, and the wide-ranging analysis involved in terms of the polarities of the compounds analysed. Both naphthodianthrones exhibited poor solubility in common solvents and in the presence of acid. In order to obtain a well-defined peak for either, the HPLC system should not contain too much water or acid. Under such conditions both compounds gave undefined, tailing peaks with varying retention times with all the columns tested. The results were the same for the extracts. This is possibly due to hypericins precipitating out in the presence of too much water at the beginning of a chromatographic run and later re-dissolving and eluting when the organic component increased. Hypericins may also form homoassociates in the presence of too much water, which have different retention times to that of the monomeric form. These may cause undefined, tailing peaks with the presence of

a pseudo-molecular ion at 503 m/z (negative ion mode). It was possible that the homoassociates disintegrated in the source as water evaporated and gave rise to peaks at m/z 503 in several regions of the chromatogram, depending on whether it was monomeric or oligomeric hypericin that was being eluted. The concentration of hypericin in the sample was also critical; the higher the concentration the more likely it is to form homoassociates in solution, which will have different retention times to mono-molecularly dissolved hypericin due to factors such as steric hindrance and differences in polarity. The above problems with hypericin meant that the analysis had to be divided into polar and lipophilic sections, whereby hypericin was not exposed to too much water inadvertently whilst analysing for more polar compounds.

The analysis of other compounds presented very few problems, even in the case of hyperforin, which is considered to be somewhat unstable.

Extraction efficiency

The extraction of pseudohypericin, quercetin, quercitrin and hyperoside was considered to be 100% efficient after 1 h. As significant amounts of hypericin, hyperforin, isoquercitrin, rutin and chlorogenic acid were found in the residue that had already been extracted for 1 h (18.3, 72.9, 11.2, 3.0 and 2.0% of the amount found after extracting for 1 h, respectively), it was further extracted for another hour and the filtrates combined. A third extraction was not done as there was very little residue remaining after the second extraction.

HPLC-ESI-MS method

Protocol 1 was established for the analysis of quercetin, its glycosides and chlorogenic acid. It is a substantial modification of the modified method described by Kartnig et al (1996).

The retention times, in minutes, were chlorogenic acid 4.0, rutin 9.9, hyperoside 12.0, isoquercitrin 12.8, quercitrin 20.5 and quercetin 34.6. Figure 2 shows a selected ion chromatogram of a sample of Kira concentrated tablets obtained using Protocol 1. The concentrations of the analytes were chlorogenic acid 27.2 $\mu g\,mL^{-1}$, rutin 148.6 $\mu g\,mL^{-1}$, hyperoside 170.8 $\mu g\,mL^{-1}$, isoquercitrin 64.6 $\mu g\,mL^{-1}$, quercitrin 18.3 $\mu g\,mL^{-1}$ and quercetin 31.3 $\mu g\,mL^{-1}$.

The analysis of a mixture of standards showed that none of the compounds influenced the chromatography of the others. Each standard was added one at a time and analysed by Protocol 1 to check for any interferences in the chromatography of the other standards. Hyperoside and isoquercitrin, which are structural isomers, could not be base resolved, but gave sufficient separation for the purposes of quantification.

Protocol 2 was developed for the analysis of hypericins and hyperforin. It is a fast and simple method for the analysis of the lipophilic compounds. The analysis of a mixture of standards showed that none of the compounds influenced the chromatography of the others. The retention times, in minutes, were hyperforin 3.3, pseudohypericin 5.2 and hypericin 8.2. Figure 3 shows a selected ion chromatogram of a sample of Boots St John's wort tablets obtained using Protocol 2. The concentrations of the analytes were hyperforin 87.4 $\mu g\,mL^{-1}$, hypericin 1.1 $\mu g\,mL^{-1}$ and pseudohypericin 2.8 $\mu g\,mL^{-1}$.

Quantification was achieved with an external standardisation method. Table 2 shows the linear range for the standards. Calibration curves were prepared using six calibration points.

Detection was with ESI-MS in the negative ionisation mode, which gave good pseudo-molecular ions for all the compounds, with little or no fragmentation under the conditions. The pseudo-molecular ion peak was the most abundant peak in the spectrum, as is characteristic of ESI-MS. The fragmentation observed was due to low energy reactions such as the loss of D-rhamnose (−146 u) from the disaccharide sugar moiety of rutin to give the de-protonated quercetin β-D-glucoside (isoquercetrin). The mass spectrum of isoquercetrin showed a peak at m/z 301, indicating the loss of the sugar moiety D-glucose from isoquercitrin to give the de-protonated aglycone quercetin (RMM 302).

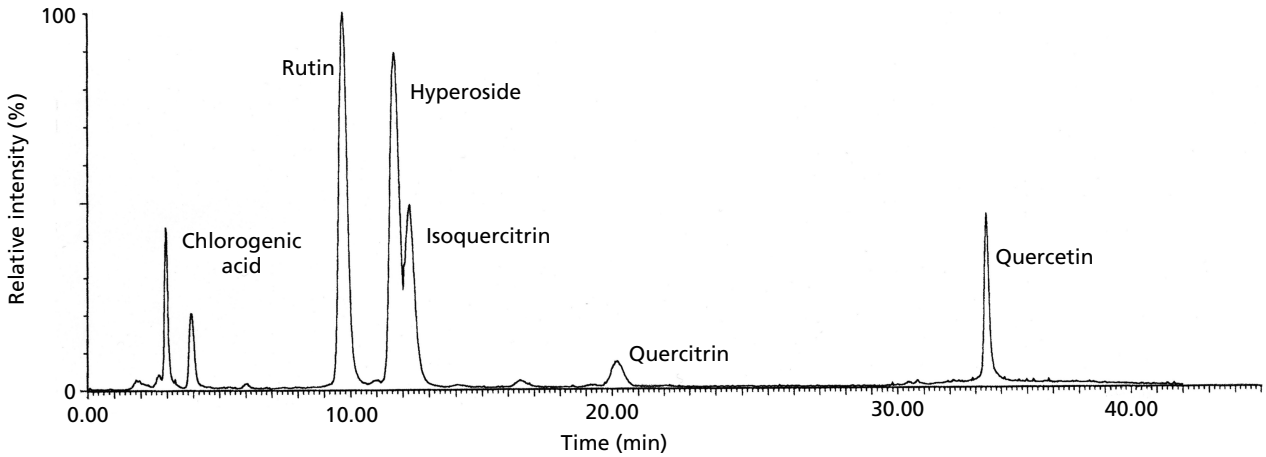

Figure 2 A selective ion chromatogram of a methanolic extract of a St John's wort product (Kira concentrated tablets), analysed using Protocol 1.

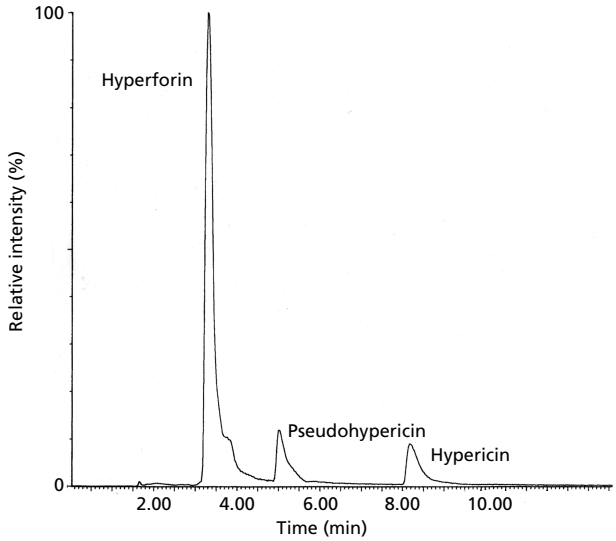

Figure 3 A selective ion chromatogram of a methanolic extract of a St John's wort product (Kira concentrated tablets), analysed using Protocol 2.

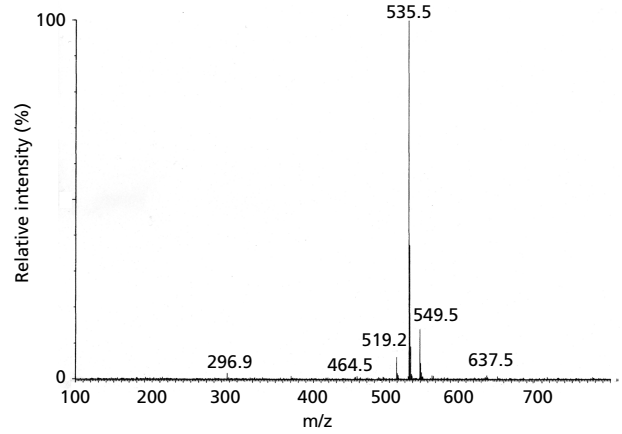

Figure 4 Negative ESI mass spectrum of hyperforin (RMM 536), acquired under the conditions stated in the Materials and Methods section.

Table 2 The linear ranges for the active constituents

Constituent	Linear range ($\mu g\,mL^{-1}$)	Regression coefficient (r)
Hyperforin	2.8–44.8	0.9711
Hypericin	0.5–22.8	0.9998
Pseudohypericin	0.1–36.0	0.9977
Quercetin	5.1–61.2	1.0000
Quercitrin	4.9–61.2	0.9994
Isoquercitrin	5.0–88.0	0.9981
Hyperoside	5.0–100.0	0.9996
Rutin	5.0–100.0	0.9994
Chlorogenic acid	5.0–55.0	0.9936

The mass spectrum of hyperforin (see Figure 4) also shows mainly the pseudo-molecular ion. The peaks at m/z 549 and 519 are most likely derived from adhyperforin and pseudohypericin, respectively, which were present as impurities.

The ESI mass spectra of hypericin and pseudohypericin did not show any obvious fragmentation patterns. The pseudo-molecular ions of these large aromatic compounds are probably very stable due to charge delocalisation and therefore unlikely to fragment under these conditions. Tandem mass spectrometric experiments performed later showed that high collisional energies were required to fragment these compounds.

Statistical treatment of data

Both protocols proved to be very specific for the constituents analysed. The analysis of a mixture of standards showed that none of the compounds influenced the chromatography of the others. Structurally similar compounds such as hypericin and pseudohypericin were resolved at baseline. Blank chromatograms showed no signals, therefore indicating the absence of false positive responses. MS analysis showed no other signals within the analyte peaks.

A low concentration linear range was established for each standard from which the limit of detection was evaluated. The limits of detection for hyperforin, hypericin, pseudohypericin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin and chlorogenic acid were 0.1, 2.0, 0.9, 19.0, 4.0, 4.0, 2.0, 1.0 and 35.0 μg on column, respectively. The limits of quantification for hyperforin, hypericin, pseudohypericin, quercetin, quercitrin, isoquercitrin, hyperoside, rutin and chlorogenic acid were 1.5, 6.0, 3.0, 65.0, 18.0, 20.0, 10.0, 4.0 and 100.0 μg on column, respectively.

The accuracy, precision and repeatability of the method were determined and the data are presented in Table 3. The intra-assay repeatability for each compound in extract analysis was also obtained.

For the analysis of extracts the intra-assay repeatability (RSD) for the compounds, based on three extract analyses and triplicate measurements, were hyperforin 0.1–9.3, hypericin 3.1–4.4, pseudohypericin 18.8–22.1, rutin 7.2–8.6, quercitrin 3.1–9.0, isoquercitrin 1.9–5.8, quercetin 1.6–5.5, chlorogenic acid 7.6–10.8 and hyperoside 13.7–17.5.

Qualitative analysis was performed by more than one operator at different times and gave reproducible data. Temperature changes had minimal effects. No specific tests were done to check robustness; however, ESI-HPLC-MS is considered to be a very robust analytical technique that is not overly sensitive to small changes in instrument parameters. Protocol 1 is a modification of the method described by Kartnig et al (1996), the reproduction of which involved testing many variations in flow rate, column, temperature, pH and the water and organic content of the mobile phase. These variations did not produce any major changes in the chromatography of chlorogenic acid or the glycosides.

Product analysis

The amounts of hypericin and hyperforin found in commercial preparations are shown in Table 4. As can be seen

Table 3 Accuracy, intra-assay variability (obtained with three standards (shown as RSDx)) and inter-day variability (obtained with six standards (shown as RSDy)) of the quantification method

Compound	Actual value ($\mu g\,mL^{-1}$)	Accuracy	RSDx	Actual value ($\mu g\,mL^{-1}$)	Accuracy	RSDy
Hyperforin	2.3	112.7	12.3	5.6	114.8	3.9
	18.1	99.5	4.6	11.2	116.3	15.9
	27.1	95.8	2.8	18.1	100.1	2.6
Hypericin	2.4	94.7	5.4	2.4	96.7	6.8
	4.6	95.9	7.3	9.5	94.1	8.4
	9.5	97.9	4.1	14.1	103.1	9.3
Pseudohypericin	4.7	98.5	5.2	4.6	95.1	7.5
	19.6	104.6	2.5	9.4	98.3	8.4
	28.3	98.1	2.8	28.3	101.6	6.3
Rutin	9.7	108.1	4.1	9.7	101.8	7.3
	19.4	94.6	3.2	19.8	104.6	2.4
	38.8	103.4	4.4	98.5	100.8	8.4
Quercitrin	5.4	98.1	5.1	9.7	97.3	4.7
	10.9	87.3	6.4	21.8	91.1	4.4
	21.8	91.6	6.8	36.7	104.7	4.6
Isoquercitrin	5.5	98.5	6.4	5.6	106.1	10.6
	7.6	87.3	0.2	22.5	99.4	8.7
	22.5	98.3	5.8	36.5	104.3	4.2
Quercetin	5.1	94.9	11.7	11.2	99.1	6.6
	10.2	104.5	1.6	20.4	99.9	6.4
	20.4	98.3	4.1	44.8	100.6	1.1
Chlorogenic acid	9.9	103.4	2.8	5.2	107.8	5.8
	19.8	95.9	2.5	9.9	101.6	1.8
	29.7	96.8	1.3	19.8	95.6	5.1
Hyperoside	5.1	102.9	6.2	4.9	100.5	6.5
	20.3	98.4	1.5	39.1	99.2	2.5
	74.5	99.8	1.1	74.5	100.2	1.5

n, number of samples; RSD, relative standard deviation.

Table 4 Quantity of hyperforin, hypericin and pseudohypericin in St John's wort products

Product	Hypericin or total hypericin as labelled (mg)	Hyperforin content per dosage form (mg ± s.d.)	Hypericin content per dosage form (mg ± s.d.)	Pseudohypericin content per dosage form (mg ± s.d.)
A (s)	0.9	12.96 ± 0.44	0.21 ± 0.05	0.72 ± 0.10
B (u)	0.9	11.03 ± 0.72	0.14 ± 0.003	0.35 ± 0.04
C (s)	0.9	7.18 ± 0.77	0.18 ± 0.005	0.70 ± 0.15
D (s)	1.05	4.21 ± 0.14	0.11 ± 0.004	0.62 ± 0.08
E (u)	0.18	0.27 ± 0.01	0.07 ± 0.002	0.27 ± 0.06
F (s)	0.95	0.14 ± 0.02	–	–
G (s)	0.45	0.35 ± 0.02	0.05 ± 0.001	0.26 ± 0.001
H (u)	Not given	0.14 ± 0.01	0.02 ± 0.001	0.11 ± 0.003
I (u)	0.3	2.66 ± 0.07	0.05 ± 0.002	0.20 ± 0.005
K (s)	0.9	0.95 ± 0.09	0.17 ± 0.004	0.43 ± 0.05
L (u)	0.3	5.74 ± 0.21	–	–

Data presented as mg per tablet or powder in capsule and expressed as mean ± s.d. of six analyses. s, standardised product; u, unstandardised product; –, not detected.

there were widely varying amounts of hyperforin in the products analysed. The daily intake could vary between 0.14 and 12.96 mg, depending on the product. The efficacy of products with very low amounts of hyperforin is questionable as it is now considered to be one of the most important constituents for the antidepressant activity of St John's wort (Chatterjee et al 1998; Laakman et al 1998). Most manufacturers standardise their products on the

basis of hypericin and pseudohypericin content although studies have not correlated the effects of the extract with the pure form of hypericin. Almost all the products evaluated, with the exception of two, would provide adequate amounts of hypericin daily (approximately 900 μg based on most manufacturers' recommended dosage). Products standardised to the same amount of hypericin showed significant variation. This variation was found to be less likely with products made by established manufacturers. Standardisation seemed to be an important factor in guaranteeing good levels of hypericin in the case of better-known products; this may be because the more established manufacturers have better methods of standardisation, manufacture and quality control. However, since we only tested one batch, this requires further comparative studies.

Widely varying flavonoid levels were found in the products (results not shown). High levels of flavonoids were found in most of the products, with a few exceptions.

Conclusion

An accurate and specific HPLC-ESI-MS method was established and validated for the extraction, separation, identification and quantification of the main active constituents of *Hypericum perforatum* L. This method proved to be robust and applicable to alcoholic tinctures, tablet/capsule extracts in various solvents and herb extracts. The HPLC analysis of *Hypericum* extracts presented specific problems due to the physico-chemical properties of the naphthodianthrones, which were rarely reported in previous literature. This will have to be taken into account when profiling St John's wort preparations.

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