ELSEVIER

Journal of Chromatography B, 735 (1999) 285–288

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Measurement of hyperforin a constituent of St. John's wort in plasma by high-performance liquid chromatography

J.D. Chi, M. Franklin*

University of Oxford, Department of Psychiatry, Psychopharmacology Research Unit, Warneford Hospital, Oxford OX3 7JX, UK

Received 7 May 1999; received in revised form 18 August 1999; accepted 31 August 1999

Abstract

Hyperforin is a constituent of *Hypericum perforatum* extracts (St. John's wort, *H. perforatum*), which have antidepressant action. Hyperforin was extracted from plasma utilising a solid-phase extraction procedure. Chromatography was performed by isocratic reversed-phase high-performance liquid chromatography with UV end-point detection. The calibration curve was linear over the range 0.15–3 μg per ml of plasma. The sensitivity for hyperforin was 4.5 ng on-column. Mean inter- and intra-assay relative standard deviations over the range of the standard curve were less than 5%. The absolute recovery for hyperforin averaged 97.8%.

Keywords: Hyperforin

1. Introduction

Hyperforin is one of the characteristic constituents of *Hypericum perforatum*. The pharmacological and therapeutic properties of *H. perforatum* have been extensively reviewed [1–7]. The structure of hyperforin is shown in Fig. 1.

Only two methods have been reported to our knowledge for the determination of hyperforin in plasma and these both appeared in the same paper [8]. The first procedure used high-performance liquid chromatography (HPLC) with UV end-point detection. The analyte was extracted from plasma by a liquid–liquid separation and was followed by evaporation of the organic phase. No mention was made of its sensitivity or recovery. The second method reported used HPLC coupled with tandem mass spectrometry (MS–MS). This method is not generally suitable for routine measurement of hyperforin in plasma because the equipment is initially costly, is expensive to maintain and requires a high degree of expertise to run.

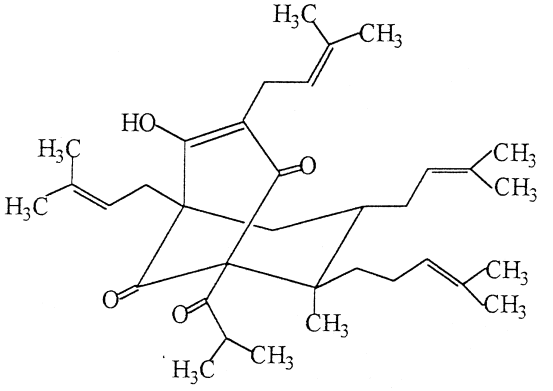

Fig. 1. Structure of hyperforin.

*Corresponding author. Tel.: +44-1865-223-137; fax: +44-1865-775-212.

0378-4347/99/$ – see front matter
PII: S0378-4347(99)00407-7

Our aim was to set up an assay procedure that was quick, simple, cheap to run, robust, gave a high degree of selectivity and could be used in a general clinical laboratory. We describe a method which we feel satisfy these criteria. The method utilises HPLC with UV end-point detection and solid-phase extraction (SPE).

2. Experimental

2.1. Materials

Hyperforin and an extract of *H. perforatum* (L1160) were kindly donated by Lichtwer Pharma (Berlin, Germany). Both orthophosphoric acid and acetonitrile were purchased from Merck (Poole, UK). All other reagents used for the assay were of the highest grade available. Water was deionised and glass distilled prior to use. Drug-free plasma for the preparation of the calibration standard was obtained from healthy volunteers.

A stock standard solution of hyperforin was prepared at a concentration of 100 μg/ml in methanol and when stored at 4°C was stable for at least two weeks. The assay standard was prepared freshly for each assay from stock solution.

2.2. Chromatography

The HPLC system consisted of a Model PU-950 pump (Jasco, Tokyo, Japan), a manual Rheodyne 7125 injection valve equipped with a 50-μl loop, a 150×4.6 mm I.D., 5 μm particle size mixed-mode (C_{18}/CN) analytical column (Capital HPLC, Edinburgh, UK) and a Model 975 UV detector (Jasco), set to the optimal wavelength of 272 nm was used for end-point detection. A Model 4400 integrator (Varian, Northants, UK) was used to analyse the chromatographic data.

The mobile phase consisted of 81% acetonitrile in water adjusted to pH 4.5 (with 1 *M* H_3PO_4) and was filtered and degassed prior to use. The flow-rate was 1.2 ml/min.

Peak height rather than peak area in the chromatograms was measured. Plasma concentration of hyperforin was assessed by interpolation of the standard curve.

2.3. Procedures

Blood samples were collected into tubes containing lithium heparin as anticoagulant. The plasma was prepared following centrifugation at 1500 *g* at 4°C and was subsequently stored at −20°C until required for assay.

Standards for assay were prepared in duplicate in 0.5 ml drug-free plasma and consisted of six concentration points over the range of 0.15–3 μg/ml and blank.

C_8 sorbent columns (50 mg Isolute, Jones Chromatography, Hengoed, UK) were conditioned with methanol and water (1 ml). The vacuum on the manifold system (VacMaster, IST, Mid-Glamorgan, UK) was diverted to prevent the columns from drying out and standards and samples were loaded. The vacuum was again applied to allow passage of materials through the column. Columns were washed with water (2×1 ml) and taken to dryness under vacuum. The vacuum was diverted and the manifold needles wiped dry. A collection tray with 75×10 mm glass tubes was inserted into the vacuum manifold and analyte was eluted with methanol (1 ml). Eluates were evaporated to dryness under vacuum at 40°C. The residue was reconstituted in 200 μl mobile phase, vortex mixed and 20 μl of solution injected into the HPLC system.

3. Results

Resolution and sensitivity was determined by injection of an extracted plasma standard (Fig. 2). The retention time of hyperforin was 7 min. The linearity of both extraction procedure and the detector response (determined from the peak height) was verified over the assay range (0.15–3 μg/ml). This was done by assaying pooled drug-free plasma (which had been previously screened for extraneous peaks) spiked with known amounts of the analyte. A calibration curve was calculated for the analyte using its concentration versus the peak height over the standard range.

The mean equation for the hyperforin calibration curve using linear regression analysis was $y=0.7499x-1.077$ ($r=0.9988$, $n=6$). The inter- and intra-assay relative standard deviations (RSDs) are

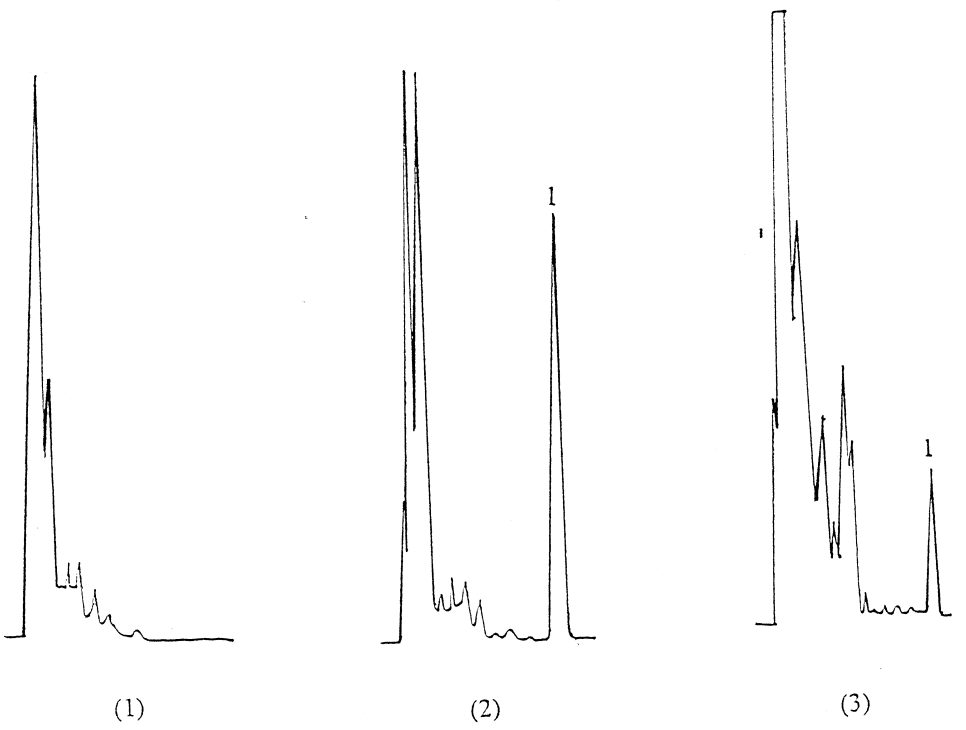

Fig. 2. Chromatogram of (1) blank drug-free plasma, (2) drug-free plasma (0.5 ml) spiked with 2.4 μg/ml hyperforin. (3) An extracted sample from a rat following an i.p. dose (200 mg/kg) of L1160 (which contained 4.3%, w/w, hyperforin) at 120 min after treatment (calculated to be 0.84 μg/ml). The retention time for hyperforin was 7 min (peak 1) with a total run time of 9 min. The injection volume was generally 20 μl on-column.

given in Table 1. The mean absolute extraction recovery for hyperforin in plasma and for QCs of 0.3, 1.2 and 2.4 μg/ml, respectively was 97.8±1.8%. (n=12). Plasma samples containing hyperforin were stable after a period of three months at −25°C and two freeze–thaw cycles. Sample extracts were stable for one week when stored out of light at 4°C.

The plasma profile of hyperforin following an i.p. injection of L1160 (200 mg/kg) extract in male rats (n=20) is shown in Fig. 3.

4. Discussion

We have described a quick, simple and highly selective HPLC assay procedure for the measurement of hyperforin in plasma. The assay detection limit

Table 1
Intra- and inter-assay precision (RSDs) and accuracy data for the determination of hyperforin in plasma

Actual value (μg/ml)	Intra-assay		Inter-assay	
	Observed value (μg/ml)[a]	RSD (%)	Observed value (μg/ml)[a]	RSD (%)
0.3	0.29±0.02	6.9	0.28±0.02	7.1
1.2	1.20±0.04	3.1	1.18±0.05	4.2
2.4	2.41±0.03	1.3	2.40±0.04	1.8

[a] The intra- and inter-assay data represents the mean±SD of 6 and 18 observations, respectively.

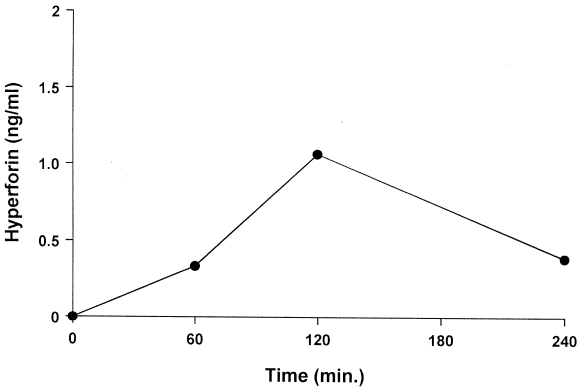

Fig. 3. Plasma concentrations of hyperforin following an i.p. injection of L1160 (200 mg/kg) in male rats (n=20).

(i.e., peak height equal to three times baseline noise) was 4.5 ng of hyperforin on column. The assay was sufficiently sensitive to determine hyperforin concentrations in plasma. A suitable internal standard for the assay was not available, however the quality control data presented adequately demonstrates that this is not actually necessary and this appears to be due to the assay's robustness and reproducibility.

The method demonstrates significant advantages with good extraction recovery, a low sample volume requirement (500 μl) and a short sample preparation time through utilisation of SPE technology. We utilised a mixed mode column for this analytical procedure because it gave us the required resolution and peak sharpness which other column packings tested failed to do. Chromatography took less than 9 min for each assay run and demonstrated good resolution of analyte without interference. The range of the standard was sufficient to incorporate the majority of expected values.

5. Conclusions

A novel, rapid and simple HPLC method has been described for the measurement of hyperforin in plasma. The assay is cheap to run and may easily be set up in a routine clinical laboratory.

References

[1] G. Laakmann, C. Schuele, M. Kieser, Pharmacopsychiatry 31 (Suppl. 1) (1998) 54–59.
[2] R. Schellenberg, S. Sauer, W. Dimpfel, Pharmacopsychiatry 31 (Suppl. 1) (1998) 44–53.
[3] S.S. Chatterjee, A. Singer, E. Mueller, Life Sci. 64 (1998) 431–437.
[4] J.A. Ascher, J.A. Cole, J.N. Cole, J. Clin. Psychiat. 56 (1995) 395–401.
[5] V. Butterweck, A. Nahrstedt, H. Winterhoff, Ann. Congress Soc. Med. Plant Res. (1998) J29.
[6] K. Linde, G. Ramirez, D. Melchart, Br. Med. J. 313 (1996) 253–258.
[7] W.E. Muller, M. Rolli, C. Schaffer, U. Hafner, Pharmacopsychiatry 30 (Suppl) (1997) 102–107.
[8] A. Biber, H. Fisher, A. Romer, S.S. Chatterjee, Pharmacopsychiatry 31 (Suppl.) (1998) 36–43.