ELSEVIER

Journal of Chromatography A, 677 (1994) 25–33

JOURNAL OF CHROMATOGRAPHY A

Qualitative and quantitative reversed-phase high-performance liquid chromatography of flavonoids in Crataegus leaves and flowers

A. Rehwald[a], B. Meier[b], O. Sticher[a,*]

[a]*Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, CH-8057 Zürich, Switzerland*

[b]*Zeller AG, CH-8590 Romanshorn, Switzerland*

First received 27 December 1993; revised manuscript received 5 April 1994

Abstract

A qualitative and a quantitative reversed-phase HPLC method for the analysis of the flavonoids in Crataegus have been developed. The qualitative fingerprint method allows the separation of the main constituents vitexin-2″-O-rhamnoside, acetylvitexin-2″-O-rhamnoside, hyperoside, vitexin, rutin and chlorogenic acid. The quantitative determination contains acid hydrolysis of the flavonoid glycosides to vitexin as main C-glycoside and quercetin as aglycone in order to reduce the complex flavonoid pattern to two major compounds. This method, applied to the quantification of several commercial samples, revealed good accuracy and precision. Compared to other, well established methods (Ph. Helv. VII, DAB 10, Ph. Franç. X), the total flavonoid content, determined as vitexin and quercetin after acid hydrolysis, was twice as high as the results obtained by applying the pharmacopoeial methods. Collected plant material (*Crataegus monogyna* Jacq.) was investigated qualitatively and quantitatively on its seasonal variations.

The HPLC fingerprint analysis can be used for qualitative analysis, especially to control identity of plant material, extracts and preparations containing hawthorn, whereas the hydrolysis method is suitable for the quantitative determination of flavonol-O-glycosides and flavone-C-glycosides with commercially available reference standards.

1. Introduction

Crataegus (hawthorn) is widely used as medicinal plant both in official and traditional medicine. It shows numerous mild, but well documented pharmacological activities [1] and few side effects. Preparations of Crataegus improve the heart function, and their indications are cases of declining cardiac performance equivalent to stages I and II of the NYHA (New York Heart Association) classification, deficiency of the coronary blood supply and mild forms of arrhythmias [2].

The flowering tops and flowers are harvested from *Crataegus monogyna* Jacq. emend. Lindm. or *Crataegus laevigata* (Poir.) D.C. (synonym: *Crataegus oxyacantha* L.) or, less frequently, from other European *Crataegus* species (Rosaceae). They contain flavonoids such as hyperoside, vitexin, vitexin-2″-O-rhamnoside and

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00345-A

acetylvitexin-2″-O-rhamnoside as well as oligomeric procyanidins and (−)-epicatechin. These two groups are considered to be the main active constituents [1,3–5], and the standardization of Crataegus leaves, flowers and preparations aims at either the flavonoids or the oligomeric procyanidins or at both.

The quantitative determination of the flavonoids by spectrophotometric analysis of the aluminium chelate complexes after acid hydrolysis, as it is required by the German and Swiss pharmacopoeias, is not very suitable for Crataegus because at least half of the total flavonoid content are C-glycosides [6] which can not be hydrolyzed by acid and therefore are not extracted with ethyl acetate and not determined. By means of a HPLC fingerprint chromatogram it could be shown in our laboratory that all the vitexin derivatives remain to a large extent in the aqueous solution which is discarded. This confirmed the results of previous works [6,7]. Besides, the prescribed wavelength of 425 nm does not represent the absorption maximum of the vitexin derivatives that have partially passed into the ethyl acetate. Since this method comprises only the flavonol-O-glycosides and not the flavone-C-glycosides, it is not accurate and yields unsatisfactory results.

Nowadays reversed-phase high-performance liquid chromatography (RP-HPLC) is the commonly used analytical separation technique for polyphenolic compounds like flavonoids. Due to the variability of column filling materials and solvent systems, RP-HPLC exhibits a great potential in separating complex mixtures of flavonoids and other phenolic compounds. Several studies on correlations between structure and retention time values of flavonoids have been carried out [8–11], using isocratic or gradient elution technique. They demonstrate the influence of the pattern of hydroxylation, degree of unsaturation and glycosylation on the chromatographic behaviour of a large number of flavonoids.

The HPLC fingerprint analysis has become a useful and suitable method for quality control and standardization of plant material and preparations. It requires good separation and resolution of the complex mixture as well as peak purity control in order to prevent peak overlapping. Davis and Giddings [12] developed a method for the evaluation of the number of components in multicomponent chromatograms, which was applied to the analysis of plant extracts by Dondi *et al.* [13].

Several authors [6,14–17] described reversed-phase HPLC fingerprint chromatograms for the quantitative determination of the flavonoids in Crataegus. They used the internal or external standard method. The greatest disadvantage of these analyses is that not all compounds are commercially available reference standards.

Therefore a quantitative reversed-phase HPLC method was developed which is based on the reduction of the complex flavonoid glycoside pattern to one major aglycone and one C-glycoside by acid hydrolysis [18]. The main flavonol-O-glycosides hyperoside and rutin can be hydrolyzed to quercetin, while the principal flavone-C-glycosides vitexin-2″-O-rhamnoside and acetylvitexin-2″-O-rhamnoside react to vitexin. Quercetin and vitexin are commercially available standards which can be used for calibration curves for the standardization of Crataegus plant material and preparations. Beside this quantitative method a fingerprint chromatogram for quality control was elaborated.

2. Experimental

2.1. Plant material

The samples of Crataegus leaves and flowers (Crataegi folium cum flore) were purchased from different commercial sources (Dixa, St. Gallen, Switzerland; Hänseler, Herisau, Switzerland). Plant extracts were provided by Zeller (Romanshorn, Switzerland). For the investigation of the seasonal variations, leaves, flowers and fruits of *Crataegus monogyna* Jacq. were collected monthly in Buchs (Canton Zürich, Switzerland) from April to October, 1993. The plant material was identified by Dr. W. Lippert, München, Germany, and voucher specimens are deposited in the Botanische Staatssammlung, München,

Germany, and in the Herbarium ETH, Zürich, Switzerland. The collected plant material was immediately dried at 35°C in a Salvis TSK 2 HL dryer (Salvis, Emmenbrücke, Switzerland) for 48 h (leaves, flowers) and for 7 days (fruits).

2.2. Standards and solvents

Quercetin dihydrate (puriss.) was from Fluka (Buchs, Switzerland); vitexin, isovitexin and vitexin-2″-O-rhamnoside (Rotichrom HPLC grade) were from Roth (Basel, Switzerland). Methanol, acetonitrile, tetrahydrofuran and isopropanol were of HPLC quality (Romil Chemicals, Shepshed, U.K.). Orthophosphoric acid (analytical-reagent grade) was purchased from Siegfried (Zofingen, Switzerland) and hydrochloric acid (analytical-reagent grade) from Fluka. Water was obtained using a NANOpure Cartridge System (Skan, Basel-Allschwil, Switzerland). Bond Elut C_{18} (500 mg) extraction columns used for sample clean-up were purchased from Analytichem International (ICT, Basel, Switzerland).

2.3. Instrumentation and column

All HPLC analyses were performed using a Hewlett-Packard instrument (Model 79994A analytical workstation, Model 1090 liquid chromatograph, Model 1040 diode-array detector). A Knauer (Berlin, Germany) prepacked column cartridge (100 × 4 mm I.D.) filled with Hypersil ODS 5 μm (Shandon, Runcorn, U.K.) was used for all chromatographic separations. A EF4 Modulyo (Edwards AG, West Sussex, U.K.) was used for freeze-drying the tea preparations.

2.4. Chromatographic conditions

Fingerprint

The mobile phase was optimized using the "PRISMA" system [19]. It consisted of solvent A [tetrahydrofuran–acetonitrile–methanol (92.4:3.4:4.2, v/v/v)] and solvent B (0.5% orthophosphoric acid). The elution profile was: 0–12 min 12% A in B, 12–25 min 12% to 18% A in B (linear gradient), 25–30 min 18% A in B. The flow rate was 1.00 ml/min, the column temperature 25.0°C and the injection volume 10 μl. The UV detector was set at 370 nm, 336 nm and 260 nm.

Determination of vitexin after acid hydrolysis

The mobile phase consisted of solvent A [tetrahydrofuran–isopropanol–acetonitrile (10:8:3, v/v/v)] and solvent B (0.5% orthophosphoric acid). The separation was carried out using isocratic elution (0–13 min 12% A in B) with a flow rate of 1.00 ml/min and a column temperature of 25.0°C. The injection volume was 5 μl, and UV detection was effected at 336 nm and 260 nm.

Determination of quercetin after acid hydrolysis

The analyses were performed by gradient elution: 0–15 min 30% A (methanol) in B (0.5% orthophosphoric acid) to 55% A in B. The flow rate was 1.00 ml/min, the column temperature 25.0°C and the injection volume 10 μl. UV detection was performed at 370 nm and 260 nm.

2.5. Sample preparation

Qualitative analysis

Fingerprint

3 g of dried and pulverized plant material or 2 g of dried plant extract were refluxed with 60 ml of methanol 80% for 60 min, filtered and refluxed with 40 ml of methanol 80% for another 10 min. The filtered extract was evaporated under vacuum to about 20 ml and diluted to 25.0 ml with 80% methanol. 5.0 ml of this solution were filtered through a Bond Elut C_{18} cartridge for sample clean-up and diluted to 10.0 ml with 80% methanol. A 10-μl volume of this solution was injected into the chromatographic system.

Quantitative analyses

Method 1. *(acid hydrolysis)*

8 g of dried and pulverized plant material or 6 g of dried plant extract were extracted in a Soxhlet apparatus with 150 ml of methanol for 5

h. The extract was evaporated under vacuum to about 80 ml and diluted to 100.0 ml with methanol. For the determination of vitexin, 25.0 ml of this solution and 10 ml of 25% hydrochloric acid were refluxed for 90 min. For the determination of quercetin, 25.0 ml of the extraction solution and 2 ml of 25% hydrochloric acid were refluxed for 60 min. After cooling the hydrolyzed extracts were diluted to 50.0 ml. 5.0 ml of these solutions were filtered through Bond Elut C_{18} cartridges for sample clean-up and diluted to 10.0 ml with methanol. A 5-μl (vitexin) and 10-μl (quercetin) volume of these solutions were injected into the HPLC system.

Method 2 (*quantitative fingerprint*)

5.0 ml of the extraction solution of method 1 were filtered through a Bond Elut C_{18} cartridge for sample clean-up and diluted to 10.0 ml with methanol. A 10-μl volume of this solution was injected into the chromatographic system.

Method 3

This method was according to Ph. Franç. X.

Method 4

This method corresponded to Ph. Helv. VII.

Tea preparation

To 1.8 g of dried plant material 150 ml of boiling water were added and stirred from time to time. After 20 min the tea was filtered, and the filtrate was freeze-dried for further investigations. The quantitative determination was effected by applying method 1.

2.6. Determination and calibration

The determination of vitexin and quercetin for method 1 was performed using the external standard method and calculating the peak areas. The calibration curves were obtained with eight samples of various concentrations using linear regression analysis. Each sample was measured three times. Over the selected concentration range of 46 μg/ml to 276 μg/ml for vitexin and 27 μg/ml to 176 μg/ml for quercetin the calibration curves showed a linear detector response. The correlation coefficients were 0.9995 for both vitexin and quercetin.

During the acid hydrolysis vitexin is partially converted into isovitexin [20] due to a Wessely–Moser rearrangement [21], and an equilibrium mixture of both isomers results. Under the conditions mentioned above, the hydrolysis mixture consists of mainly vitexin and approximately 6% isovitexin. Isovitexin was calculated as vitexin by summing the peak areas. There was no significant difference ($p < 0.001$) between this calculation and the determination with an additional calibration curve for isovitexin.

The quantitative determination of vitexin-2″-O-rhamnoside and hyperoside for method 2 was carried out with external standards (six-point calibration). The correlation coefficients were 0.9993 for vitexin-2″-O-rhamnoside and 0.9990 for hyperoside. Acetylvitexin-2″-O-rhamnoside was calculated as vitexin-2″-O-rhamnoside and rutin as hyperoside.

For method 3 the quantitative determination of vitexin-2″-O-rhamnoside and hyperoside was performed using external standards (one-point calibration).

2.7. Reproducibility

The reproducibility of the chromatographic separations was verified with columns from several batches. The fingerprint method and the determination of quercetin after acid hydrolysis were reproducible on five different column batches, the separation of vitexin, isovitexin and vitexin-2″-O-rhamnoside for the determination of vitexin after acid hydrolysis was reproducible on three columns. All chromatographic analyses were reproducible on a second HPLC instrument (Hewlett-Packard HPLC^{3D} ChemStation, 1090 LC Series II).

3. Results and discussion

3.1. Extraction and sample preparation

The preparation of the plant material was performed by continuous, exhausting extraction

in a Soxhlet apparatus. Other extraction methods like reflux or turbo extraction, which are proposed by various authors [6,14,18] were tested, but they revealed only incomplete extraction of the flavonoids, even if repeated several times. The resulting flavonoid content was at least 15% lower.

The hydrolytic conditions for the flavonoid glycosides were examined in a first step using reference standards (hyperoside and vitexin-2″-O-rhamnoside), subsequently transferred to the plant material and finally optimized. For the hydrolysis of hyperoside and other flavonol-O-glycosides mild hydrolytic conditions are sufficient. The vitexin derivatives vitexin-2″-O-rhamnoside and its acetate require more hydrochloric acid and a longer time of refluxing for a complete hydrolysis. These conditions again are too strong for the flavonol-O-glycosides and lead to a loss of 15–20% of quercetin. Similar results were found by other authors [22] who report a significant degradation of 10–20% of quercetin due to an increased acid concentration and an increased reaction period. The fact that quercetin is partially decomposed by the acid and by the heat might also explain the inferior recovery of quercetin and hyperoside compared to vitexin and vitexin-2″-O-rhamnoside (see below). Therefore these two groups of glycosides are hydrolyzed and analyzed separately.

3.2. Qualitative analysis

Five commercial samples (Crataegi folium cum flore) were examined for their flavonoid composition. They all showed rather uniform fingerprint chromatograms with vitexin-2″-O-rhamnoside, hyperoside and acetylvitexin-2″-O-rhamnoside as main peaks in similar relative concentrations. Beside little amounts of vitexin and rutin, chlorogenic acid was detected in all samples (see Fig. 1). In contrast to that the collected plant material (*Crataegus monogyna* Jacq.) contained no acetylated vitexin-2″-O-rhamnoside. Lamaison *et al.* [14] obtained contrary results. They found the acetylated vitexin-2″-O-rhamnoside only in the leaves of *Crataegus monogyna*, but not in *Crataegus laevigata*.

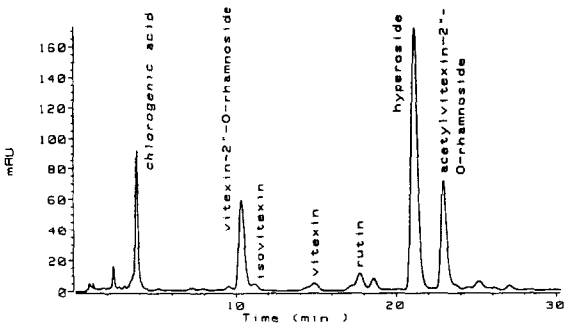

Fig. 1. Typical HPLC fingerprint chromatogram of hawthorn leaves with flowers. For conditions, see Experimental.

In all samples of collected plant material we could detect vitexin-2″-O-rhamnoside, hyperoside, vitexin, rutin and chlorogenic acid, but, however, considerable differences concerning the relative concentrations could be made out, depending on the part of the plant and on the stage of development. In the flowers (collected in the beginning of May) we found, corresponding to other authors [6,7,14], hyperoside as main constituent, whereas vitexin-2″-O-rhamnoside was predominant in the leaves over the whole collecting period. The wooden branches exhibited flavonoid patterns similar to the leaves, but at low concentrations and with relatively more hyperoside. The fruits (collected from August to October) revealed mainly hyperoside and notable amounts of rutin, but almost no vitexin-2″-O-rhamnoside. Also the flowers showed a remarkable amount of rutin, while in the leaves it was, as well as vitexin, present only in minor concentrations.

The drying of the collected plant material had no influence on the flavonoid composition. We found no qualitative differences and the same relative concentrations in the fingerprint chromatograms of dried and fresh plant material.

3.3. Quantitative analysis

Five commercial samples (Crataegi folium cum flore) were quantified by the hydrolysis method described above in order to obtain some information on its precision and accuracy. The relative standard deviations ($n = 3$ or 6) were

between 1% and 3% in all samples. The recoveries were determined by twice adding two different amounts of each vitexin and vitexin-2″-O-rhamnoside and two different amounts of quercetin and hyperoside at the beginning of the sample extraction. For vitexin-2″-O-rhamnoside and hyperoside the theoretically expected amounts of vitexin and quercetin, assuming a complete hydrolysis, were calculated. The recoveries were, on an average, 96.4% for vitexin and 91.5% for quercetin. This indicates almost complete hydrolysis and only little loss of compounds during the sample preparation.

Twelve batches of dried leaves, flowers and fruits of *Crataegus monogyna* Jacq. at different plant development stages were examined for their flavonoid content using method 1 (see Fig. 2). The highest amount of total flavonoids was found in the leaves during the pre-flowering time (April) and the flowering time (beginning of May). Afterwards the total flavonoid content in the leaves decreased and remained at a rather constant level, comparable with the flowers. The fruits, which contain mainly oligomeric and polymeric procyanidins [23], exhibited very low flavonoid concentrations, so that their flavonoid determination and standardization could be left out. The flowers and leaves revealed remarkable

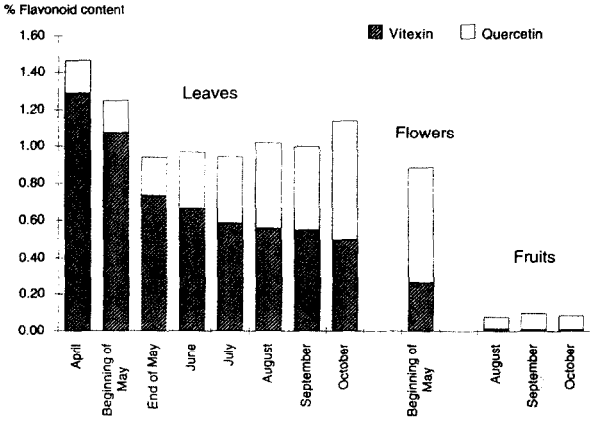

Fig. 2. Flavonoid determination by the hydrolysis method of *Crataegus monogyna* Jacq. at different stages of development.

differences concerning the ratio of vitexin and quercetin: the ratio was 0.4 in the flowers, but 7 in the leaves at the beginning of the collecting period, decreasing to 0.8 in October.

These results are in accordance with the results of other authors who report the maximal flavonoid content for the leaves during the pre-flowering and the flowering time in May [23,24], with an increasing amount of flavonol-O-glycosides towards the end of the vegetation period [24]. Taking into consideration the obtained results, it is recommendable to harvest both the leaves and flowers during the flowering time to get on the one hand the highest content of total flavonoids and, on the other hand, a well-balanced proportion of flavonol-O- and flavone-C-glycosides.

With the intention to get some information on the validity of the hydrolysis method, it was compared to three other quantitative methods (methods 2–4). Two commercial samples 1 and 2 (Crataegi folium cum flore) were quantified by these four methods. The results are represented in Table 1. Statistical calculations were carried out by means of the two-tailed Student's t-test.

In both samples the total flavonoid amount obtained by method 2 was 6–6.5% higher (significant; $p < 0.001$) than by method 1. We also found differences concerning the relative concentrations of flavonol-O-glycosides and flavone-C-glycosides : the ratio of vitexin-2″-O-rhamnoside to hyperoside was 1.9 with method 1, but 2.8 with method 2. These differences were significant ($p < 0.001$).

Methods 1 and 2 consider the whole spectrum of flavonoids, that is to say the flavonol-O-glycosides and the flavone-C-glycosides and yield similar amounts of total flavonoids. Nevertheless, method 1 should have priority, because after a simple hydrolysis procedure in order to reduce the flavonoid pattern to two compounds, it is possible to perform the quantitative determination with only two reference standards, whereas with a fingerprint method (like 2) more reference substances and calculating factors are needed.

The results obtained with method 3 were

Table 1
Quantitative flavonoid determination of two samples by four different methods

Method	Flavonoid content (%, dry weight)					
	Sample 1			Sample 2		
	VR [a]	H [b]	Σ [c]	VR [a]	H [b]	Σ [c]
Method 1 [d] (acid hydrolysis)	0.957 (1.9)	0.503 (2.3)	1.460 (1.9)	0.885 (3.2)	0.460 (2.0)	1.345 (2.4)
Method 2 (fingerprint)	1.130 (2.0)	0.415 (3.5)	1.545 (2.3)	1.065 (1.8)	0.372 (4.1)	1.436 (2.3)
Method 3 (Ph. Franç. X)	0.493 (8.2)	0.289 (4.1)	0.783 (6.0)	0.341 (2.0)	0.359 (3.5)	0.700 (1.6)
Method 4 (Ph. Helv. VII)			0.734 [e] (7.0)			0.721 [e] (7.3)

[a] Mean ($n = 6$) content of vitexin-2″-O-rhamnoside (VR) with relative standard deviation (%) in parentheses.
[b] Mean ($n = 6$) content of hyperoside (H) with relative standard deviation (%) in parentheses.
[c] Total flavonoid content, obtained by summing vitexin-2″-O-rhamnoside and hyperoside.
[d] To compare the results with those of methods 2 and 3, vitexin was calculated as vitexin-2″-O-rhamnoside and quercetin as hyperoside.
[e] Mean ($n = 6$) of total flavonoid content with relative standard deviation (%) in parentheses.

clearly lower, although this method is, in principal, similar to method 2. The significant ($p < 0.001$) loss of about 30% of hyperoside compared to methods 1 and 2 is mostly due to the incomplete extraction of the plant material. The significant ($p < 0.001$) decrease of 50–60% of vitexin-2″-O-rhamnoside compared to methods 1 and 2 resulted from the non-consideration of the acetylated vitexin-2″-O-rhamnoside. Since this compound is not commercially available, an inserted hydrolysis step or summing the peak areas of vitexin-2″-O-rhamnoside and acetyl-vitexin-2″-O-rhamnoside could easily include it.

Method 4, which determines the total flavonoid content, calculated as hyperoside, revealed results in the range of those of method 3. It is, as criticized often [6,7,15,18], not specific and neglects the flavone-C-glycosides. It should not be applied to herbal drugs with a considerable content of flavone-C-glycosides.

The obtained results are in agreement with those reported by other authors: Lamaison *et al.* [15] found in samples of Crataegi folium cum flore 1.74% of total flavonoid content by means of an HPLC fingerprint analysis, but only 0.91% with the spectrophotometric determination. Applying the method of Ph. Franç. X they obtained 0.56% of hyperoside and 0.54% of vitexin-2″-O-rhamnoside. Wagner *et al.* [6] determined 1.87% of total flavonoids in Crataegi folium cum flore by HPLC fingerprint analysis and 1.20% by spectrophotometric quantification.

A tea preparation, according to the prescription in the experimental part which is based on literature [25, 26], was quantified by method 1. From the given single dose of 1.8 g plant material (which is prescribed 2–3 times daily), 10–15 mg flavonoids, calculated as hyperoside, were extracted by hot water. This was only half as much as could be extracted by an exhausting extraction with methanol, but it is, already in a single dose, more than the required minimal

daily dose of 10 mg total flavonoids, calculated as hyperoside [2]. The tea preparation was additionally examined for the qualitative composition. It revealed exactly the same fingerprint chromatogram as an extract with 80% methanol.

4. Conclusions

The flavonol-O-glycosides and the flavone-C-glycosides seem to exhibit qualitatively and quantitatively different pharmacological activities [27]. Concerning the inhibition of the 3′,5′-cyclic adenosine monophosphate phosphodiesterase, hyperoside as flavonol-O-glycoside showed a clearly higher potency than the flavone-C-glycosides vitexin, vitexin-2″-O-rhamnoside and acetylvitexin-2″-O-rhamnoside [28,29]. C-glycosides are generally considered to be poorer inhibitors than O-glycosides [29,30], and flavonols exhibit a higher inhibitor potency than flavones [31]. On the other hand, for various flavones and flavone-C-glycosides (among them vitexin) an antiarrhythmic and antiischaemic activity was demonstrated [32]. A positive inotropic effect and an increase of the coronary blood flow was revealed by vitexin-2″-O-rhamnoside, but not by hyperoside [33]. Taking this aspect into consideration, it is certainly advantageous to have the possibility of determining flavonol-O-glycosides and flavone-C-glycosides separately. Therefore we consider acid hydrolysis of the flavonoids to quercetin and vitexin as the most convenient method nowadays for the quantitative analysis of flavonoids in hawthorn as well as for the standardization of hawthorn extracts and preparations.

Acknowledgements

Financial support from Ciba-Geigy OTC Pharma Schweiz, Gland, Switzerland is gratefully acknowledged. We also thank Dr. W. Lippert, München, Germany, for kindly identifying the collected plant material.

References

[1] H.P.T. Ammon and M. Händel, *Planta Med.*, 43 (1981) 105, 209, 313.
[2] *Bundesanzeiger*, Nr. 1, 3. 1. 1984.
[3] A. Leukel, U. Fricke and J. Hölzl, *Planta Med.*, 52 (1986) 545.
[4] A. Leukel-Lenz, *Thesis*, Philipps-Universität, Marburg, 1988.
[5] C. Roddewig and H. Hensel, *Arzneimittel-Forsch.*, 27 (1977) 1407.
[6] H. Wagner, G. Tittel and S. Bladt, *Dtsch. Apoth.-Ztg.*, 123 (1983) 515.
[7] H. Glasl, *Fresenius' Z. Anal. Chem.*, 321 (1985) 325.
[8] L.W. Wulf and C.W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
[9] K. Vande Casteele, H. Geiger and C. Van Sumere, *J. Chromatogr.*, 240 (1982) 81.
[10] D.J. Daigle and E.J. Conkerton, *J. Chromatogr.*, 240 (1982) 202.
[11] M.C. Pietrogrande, P. Reschiglian, F. Dondi, Y.D. Kahie and V. Bertolasi, *J. Chromatogr.*, 592 (1992) 65.
[12] J.M. Davis and J.C. Giddings, *Anal. Chem.*, 55 (1983) 418.
[13] F. Dondi, Y.D. Kahie, G. Lodi, M. Remelli, P. Reschiglian and C. Bighi, *Anal. Chim. Acta*, 191 (1986) 261.
[14] J.L. Lamaison and A. Carnat, *Pharm. Acta Helv.*, 65 (1990) 315.
[15] J.L. Lamaison, A. Carnat, C. Petitjean-Freytet and A.P. Carnat, *Plant. Med. Phytother.*, 25 (1991) 177.
[16] P. Ficarra, R. Ficarra and A. Tommasini, *Farmaco Ed. Prat.*, 39 (1984) 148.
[17] A. Hiermann and T. Kartnig, *Sci. Pharm.*, 52 (1984) 30.
[18] A. Hasler, *Thesis*, No. 9353, ETH Zurich, 1990.
[19] Sz. Nyiredy, B. Meier, C. Erdelmeier and O. Sticher, *J. High Resol. Chromatogr. Chromatogr. Commun.*, 8 (1985) 186.
[20] J.B. Harborne, T.J. Mabry and H. Mabry, *The Flavonoids*, Chapman and Hall, London, 1975.
[21] F. Wessely and G. Moser, *Monatsh. Chem.*, 56 (1930) 97.
[22] M. Hertog, P. Hollman and D. Venema, *J. Agric. Food Chem.*, 40 (1992) 1591.
[23] T. Kartnig, A. Hiermann and S. Azzam, *Sci. Pharm.*, 55 (1987) 95.
[24] J.L. Lamaison and A. Carnat, *Plant. Med. Phytother.*, 25 (1991) 12.
[25] *Standardzulassungen für Fertigarzneimittel (1986)*, Deutscher Apothekerverlag, Govi Verlag, Frankfurt, Monographie Stand 12, March 1986.
[26] M. Wichtl, *Teedrogen*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1989.
[27] M. Schüssler and J. Hölzl, *Dtsch. Apoth.-Ztg.*, 132 (1992) 1327.
[28] M. Schüssler, U. Fricke, N. Nikolov and J. Hölzl, *Planta Med.*, 57, Suppl. Issue 2 (1991) A133.

[29] T. Nikaido, T. Ohmoto, U. Sankawa, T. Tomimori, Y. Miyaichi and Y. Imoto, *Chem. Pharm. Bull.*, 36 (1988) 654.
[30] E. Petkov, N. Nikolov and P. Uzunov, *Planta Med.*, 43 (1981) 183.
[31] A. Beretz, R. Anton and J.C. Stoclet, *Experientia*, 34 (1978) 1054.
[32] F. Occhiuto, G. Busa, S. Ragusa and A. De Pasquale, *Phytotherapy Research*, 5 (1991) 9.
[33] F. Occhiuto, C. Circosta, R. Costa, F. Briguglio and A. Tommasini, *Plant. Med. Phytother.*, 20 (1986) 52.