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High-performance liquid chromatographic determination of oligomeric procyanidins from dimers up to the hexamer in hawthorn

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Abstract

An HPLC method using UV diode array detection was developed for analysing procyanidins qualitatively and quantitatively up to the hexameric level in hawthorn samples. The analysed compounds included procyanidin dimers **B-2**, **B-4** and **B-5**, procyanidin trimers **C-1**, epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin and epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin, a tetramer **D-1** and a pentamer **E-1** both consisting of (−)-epicatechin units linked through **C-4**β/**C-8** bonds. The concentrations of two unknown tetramers and a hexamer **F** were also quantified. The oligomeric procyanidins (OPs) were specifically determined due to the development of a method for isolating them from hawthorn during sample preparation. The pattern of oligomeric procyanidins in the leaves, flowers and fruits was similar, but the concentrations varied depending on the part of the plant. The concentration in leaves was 1.6%, in flowers 1.2% and in fruits 0.2% of the dry mass. The method was validated with respect to repeatability, recovery, linearity, and sensitivity. The repeatability for the quantitative analytical method of all the OPs in leaves was 7.7%, in flowers 8.8%, and in fruits 12.3%. The recovery of the main OPs ranged from 91 to 97%. The correlation coefficients of calibration curves were between 0.997 and 1.000. The limits of quantitation for different procyanidin standards were 0.05–0.12 mg/ml, when 10 μl of each standard solution was injected into the HPLC.

Keywords: *Crataegus laevigata*; Hawthorn; Procyanidins; Tannins; Polyphenols

1. Introduction

Hawthorn (*Crataegus* sp.) extracts have beneficial effects on the heart and blood circulation including cardiovascular protective and hypotensive effects [1–3]. Oligomeric procyanidins are considered to be the

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main active constituents, in addition to flavone- and flavonol-type flavonoids [4]. Procyanidins or condensed tannins including flavan-3-ol units, epicatechins and/or catechins (Fig. 1), can be categorized as oligomeric procyanidins (OPs) consisting of 2–6 flavanol units, and polymeric procyanidins (PPs) consisting of more than six flavanol units [5]. The presence of procyanidins up to the hexameric level has been reported in hawthorn extracts [6].

Procyanidins are widespread in nature as secondary metabolites [5], and they have also been found in commonly consumed foods [7]. Polyphenolic procyanidins have received attention owing to their antioxidant and radical scavenging activities [5], while polyphenolics with a low degree of polymerization increase coronary flow and cardiac contractility, and decrease blood pressure [2]. Many of the properties of procyanidins are based on their ability to form complexes with proteins and polysaccharides. They can undergo both intra- and intermolecular hydrogen bondings [5].

The standardization of hawthorn plant material and preparations is performed by determining the concentrations of either the flavonoids or oligomeric procyanidins [4]. Procyanidins are determined by colorimetric methods as cyanidinchloride [8] or as phenols [9], but these methods do not reveal the concentrations of individual oligomers but total concentrations. Rohr et al. [10] developed a high-performance liquid chromatographic (HPLC) method for the quantitative analysis of procyanidin dimers (**B-2**, **B-4** and **B-5**) and procyanidin trimer C-1 in hawthorn leaves and flowers. However, larger procyanidins have not been quantified. In contrast to the case for flavonoids [11], the procyanidin content in *Crataegus* sp. is mainly unknown.

There is considerable interest in the procyanidin contents of plants and foods, but the analyses are difficult to perform due to the structural diversity of OPs and the lack of procyanidin standard substances commercially available. Determinations of individual OPs, including dimers and trimers, have mainly been performed by HPLC under reversed-phase conditions [10,12–14]. Procyanidins have also been separated according to their degree of polymerization, without actually separating individual compounds, by normal-phase HPLC–mass spectrometry (MS) [15,16]. However, HPLC–MS has not proved suitable for the quantitative determination of OPs in hawthorn, because ionization of the OPs under the same experimental conditions is different. Therefore, an unresolved question is, which ion should be selected for the quantitative analysis [6]. Quality control and standardization of hawthorn preparations and plant material have been found to be inadequate because the structures and concentrations of OPs in hawthorn are largely not known. Physiological activities of individual compounds in relation to their polymerization degrees are also unknown. Therefore, suitable methods for determining individual procyanidins are needed. The main objective of this study was to develop an HPLC method using UV diode array detection for determining OPs in hawthorn plant material and raw extracts in order to obtain detailed information on the oligomeric procyanidin profiles and the concentrations of oligomers up to the hexamer present in hawthorn.

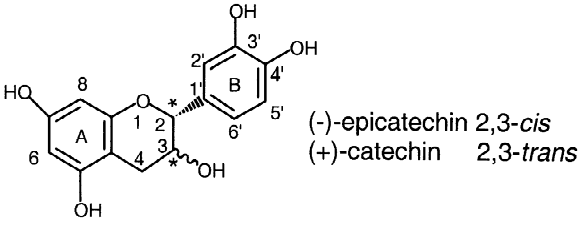

Fig. 1. Flavan-3-ol.

2. Experimental

2.1. Materials

The dried flowers, leaves and fruits of hawthorn (*Crataegus laevigata*) were kindly donated by Flachsmann, Germany. The oligomeric procyanidins used in this work were isolated from the leaves and flowers of hawthorn, and the structures of the isolated procyanidins were determined by means of spectroscopic and chemical hydrolysis methods [17]. The isolated compounds included procyanidin dimers epicatechin-(4β→8)-epicatechin (**B-2**), catechin-(4α→8)-epicatechin (**B-4**), epicatechin-(4β→6)-epicatechin (**B-5**), procyanidin trimers epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (**C-1**), epicatechin - (4β→8) - epicatechin - (4β→6) - epicatechin

(trimer II), epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin (trimer III), a tetramer (**D-1**) and a pentamer (**E-1**) both of them consisting of (−)-epicatechin units linked through **C-4**/**C-8** bonds. Two unknown tetramers and a fraction containing a hexamer (**F**) were also isolated. Acetonitrile and methanol were of HPLC grade from Rathburn (Walkerburn, UK), ethanol (96%, v/v) from Primalco (Finland) and methanol (technical grade) from Neste Resins (Hamina, Finland). Acetic acid 99.8%, acetone and light petroleum (b.p. 40–60 °C) were analytical grade from Riedel-de Haën (Seelze, Germany). Water was obtained using the Milli-Q plus purification system Millipore (Molsheim, France).

2.2. High-performance liquid chromatography

The HPLC system consisted of a Waters 600E Multisolvent Delivery System, autosampler 717, programmable photodiode array detection (DAD) system 991 (Millipore, USA) coupled to a Nec Power Mate 386/25 personal computer (USA). Solvent A was 2.5% aqueous acetic acid, and solvent B was acetonitrile–2.5% aqueous acetic acid (80:20, v/v). The linear gradients were: solvent B 7–20% in 50 min, 20–40% B from 50 to 60 min, 100% B from 60 to 65 min, followed by washing and reconditioning the column. The flow-rate was 1 ml/min, detection at 279 nm, column: LiChroCart, 250-4, Hypersil ODS (5 μm), Merck.

2.3. Sample preparation

A 0.500 g amount of milled leaves, flowers or fruits was extracted with a mixture of methanol (pH 6.5) and water (7:3), first with 20 ml and then with 15 ml, in an ultrasonic bath for 15 min, after which the mixture was centrifuged at 3000 rpm for 10 min. The sediment was then extracted with 10 ml of methanol in an ultrabath for 15 min, and after centrifugation, the sediment was finally washed with 5 ml of methanol. The resulting supernatants were filtered and extracted to eliminate chlorophyl and lipophilic compounds with light petroleum (3×30 ml). The extract was evaporated to dryness and dissolved in 1 ml of methanol–water (1:1). This was adsorbed on the top of a polyamide column prepared by mixing 0.5 g of Polyamid CC 6 for column chromatography, grain size 0.05–0.16 mm (Macherey Nagel, Düren, Germany) and 25 ml of methanol. The mixture was poured into a chromatography column which was a disposable syringe of 5 ml. The column was first eluted with methanol (pH 6.5) (2×7 ml) to give fractions **P1** and **P2**, and then with 20 ml of acetone–water (7:3) to give fraction **P3**. Fraction **P2** was evaporated to dryness, dissolved in 0.5 ml of ethanol and adsorbed on a Sephadex LH-20 column prepared by mixing 1 g of Sephadex LH-20 with ethanol on the previous day. The gel was poured into a chromatography column (55×9 mm I.D.). The column was eluted with ethanol, first with 10 ml and then with 15 ml, and two fractions, **S1** and **S2**, were collected. The same column was used for the preparation of all the samples. Fractions **S2** and **P3** were combined and the solvents were evaporated under reduced pressure. The residue was dissolved in 1.0 ml of methanol and filtered (Spartan 13, 0.45 μm, Schleicher & Schuell, Dassel, Germany), and 10 μl of sample solution was analysed by HPLC.

2.4. Calibration

Standard solutions for the HPLC analysis of OPs were prepared by dissolving isolated OPs in methanol as individual solutions in the concentration range of 0.05–4.00 mg/ml. The calibration graphs of the main OPs were constructed using five different concentrations by plotting the peak area versus the procyanidin concentration. All standard solutions were filtered (0.45 μm, Schleicher & Schuell), and 10 μl of each solution was injected into the HPLC.

2.5. Recovery studies

The recovery was determined by adding known amounts of the main OPs to three flower samples prior to extraction, and the samples were prepared as described in Section 2.3. The known concentrations of OPs were prepared by dissolving 1.024 mg/ml of dimer **B-2**, 1.200 mg/ml of trimer **C-1**, 0.244 mg/ml of tetramer **D-1**, and 0.528 mg/ml of pentamer **E-1** in methanol, and 250 μl of these solutions were added to samples. The determined concentration of each oligomer was divided by the added concen-

tration, and the recoveries were calculated as percentages. The added concentrations were based on mean concentrations of these OPs in three unspiked samples.

3. Results and discussion

3.1. Preparation of the sample solution

The hawthorn extracts contain, in addition to OPs, various phenolic compounds such as phenolic carboxylic acids, flavonoid glycosides and polymeric procyanidins. Because it was not possible to analyse all the compounds in one HPLC run, the phenolic compounds were fractionated. Fraction **P3** contained oligomeric procyanidins from dimers up to hexamer. Part of the dimeric procyanidins were eluted together with the flavonoid glycosides and phenolic acids in fraction **P2**, and the dimers were then isolated in fraction **S2**. The OPs in fractions **P2**, **P3** and **S2** were identified by TLC and HPLC–DAD analyses using isolated compounds as reference substances.

The yield of OPs is strongly affected by the method used to extract them from plant material. The solvents used for the extraction of OPs include ethanol, methanol, water, acetone, or different combinations of these solvents. Procyanidins were extracted from tea, coffee and soluble cacao with water [18], and from Spanish foodstuffs with methanol [18]. Grape seeds were extracted in a stepwise manner with methanol, and aqueous methanol and acetone [14]. Procyanidins were obtained from hawthorn by extracting with 45% (v/v) aqueous ethanol [9], and with 70% (v/v) aqueous acetone [8,10,19] as well as from various foods [15], and cranberries and blueberries [16]. Extraction of the procyanidins from hawthorn plant material with a mixture of methanol–water (7:3) proved to be the most efficient compared to that by ethanol or methanol extraction. Evaporation of acetone from an extraction mixture of acetone–water (7:3) caused precipitation, reported also by other authors [10,19]. The total quantity of OPs was obtained from hawthorn plant material by three successive extractions. The first extraction yielded 49% of OPs for flowers and 59% for leaves, the second extraction yielded 44 and 35% of OPs, respectively, and the third one 7% for flowers and 6% for leaves. OPs were not detectable in the fourth extraction.

3.2. Separation and identification

The samples were found to contain a complex series of oligomeric procyanidins. The eluted peaks were identified using previously isolated OPs as reference substances. Three dimers, trimers and tetramers, one pentamer and one hexamer were identified from the samples prepared from leaves and flowers of hawthorn (Fig. 2). There were also some unidentified peaks on the HPLC chromatograms, and they were assumed to be procyanidins on the basis of their UV spectra.

The main OPs (**B-2**, **C-1**, **D-1**, **E-1** and **F**) eluted in order of increasing degree of polymerization. However, the 4→6 linkage had an influence on the elution order. Trimer III, with the upper and middle (−)-epicatechin units linked together by a 4→6 bond, was the first of the trimers to be eluted, and occurred before dimer **B-2** (peak 2, in Fig. 2). Trimer II, possessing a 4→6 linkage between the lower and middle units, was the last of the three trimers and also of all the isolated OPs to be eluted (peak 17, in Fig. 2). Dimer **B-5** with a 4→6 linkage had the longest retention time of the dimers. This elution order is also in agreement with the results reported by Treutter et al. [12]. Dimer **B-4**, containing (+)-catechin as the upper unit of the molecule, had the shortest t_R of all the OPs (peak 1, in Fig. 2).

Suitable solvents for the HPLC separation of procyanidins on a reversed-phase column are methanol or acetonitrile, together with an acidic aqueous solution [10,12–14]. The OPs of hawthorn were preliminarily separated using a reversed-phase column, LiChrospher RP 8. The mobile phase consisted of 2.5% aq. acetic acid and methanol–2.5% aq. acetic acid (80:20, v/v) in a linear elution gradient. The separation of the OPs obtained with different methanol gradients was not satisfactory. Replacing methanol with acetonitrile gave good baseline separations of the OPs and good peak shapes. The individual OPs were separated on HPLC using 2.5% aq. acetic acid and acetonitrile–2.5% aq. acetic acid (80:20, v/v) as the mobile phases in a linear elution gradient. Acetic acid in the mobile phase suppressed

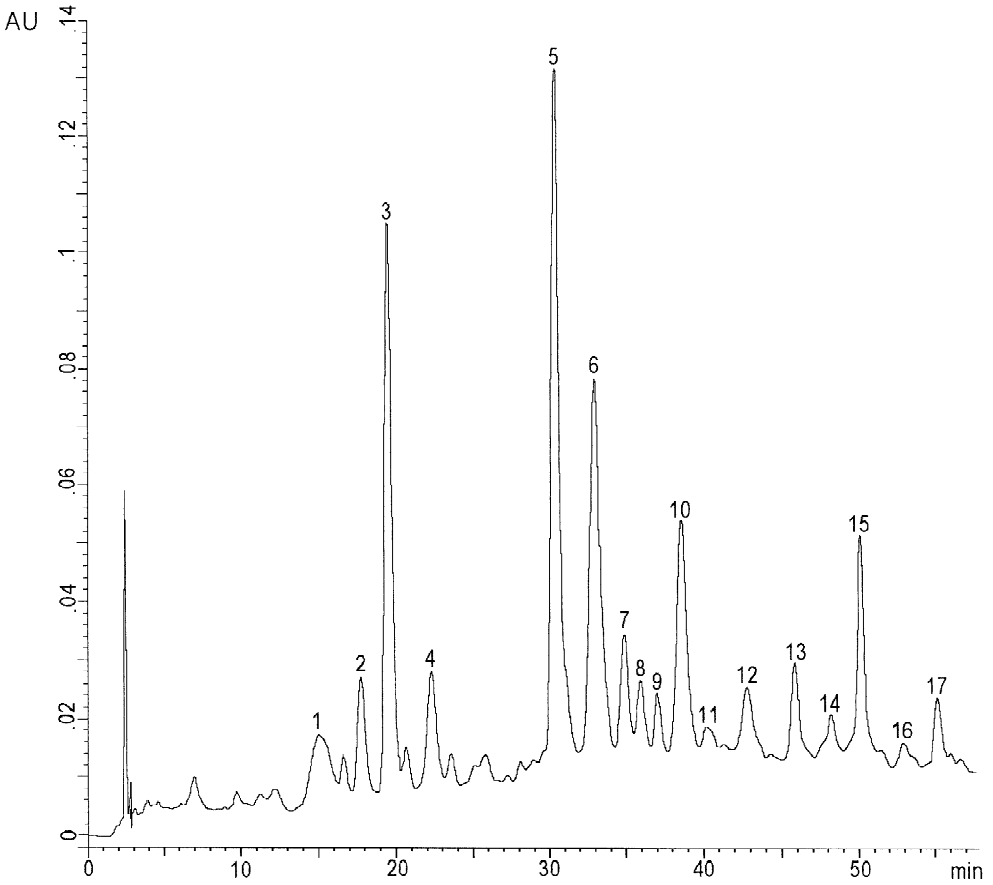

Fig. 2. An HPLC chromatogram of a sample prepared from the leaves of *C. laevigata*. The peaks correspond to the compound numbers in Table 1. Solvent A 2.5% aq. acetic acid, solvent B acetonitrile–2.5% aq. acetic acid (80:20, v/v). Linear gradients: solvent B 7–20% in 50 min, 20–40% B from 50 to 60 min, 100% B from 60 to 65 min. Flow rate was 1 ml/min, detection at 279 nm, column LiChroCart, 250-4, Hypersil ODS (5 μm).

the ionization of the phenolic procyanidins. A reversed-phase RP 18 column separated the OPs better than the RP 8 column (Table 1).

3.3. Method validation

The method was validated with respect to repeatability in peak area, linearity, sensitivity (limit of quantitation), and recoveries for the main OPs (**B-2**, **C-1**, **D-1** and **E-1**). Five-point standard curves were generated for the main OPs covering the entire range of expected concentrations of the compounds to be quantified. The correlation coefficients (r) for calibration curves varied from 1.000 to 0.997 (Table 2), indicating good linearity for all the OPs. The limits of quantitation 0.05–0.12 mg/ml for different oligomers were determined experimentally. The recovery, expressed as the mean of three replications, was 92% for procyanidin dimer **B-2**, 91% for trimer **C-1**, 97% for tetramer **D-1**, and 95% for pentamer **E-1**, indicating good accuracy of the method. The repeatability of the method was determined with six parallel sample solutions prepared from milled dried leaves, flowers and fruits of hawthorn to test the repeatability of the overall method including extraction. The repeatability of the analytical method was calculated from the mean value of the RSD of the individual OPs. The average repeatability for the quantitative analytical method of all the OPs in leaves was 7.7%, in flowers 8.8%, and in fruits 12.3% (Table 1).

Table 1
Concentrations of oligomeric procyanidins in Fig. 2 (μg), in the leaves and flowers of hawthorn (% of dry mass), retention times (t_R) in minutes, and RSD%, $n=6$

Peak No.	t_R (min)	Compound	Fig. 2[a] (μg)	% Dry mass (%) (RSD, %)	
				Leaves	Flowers
1	15.0	C(4α→8)E **B-4**	1.41	0.03 (8.48)	0.01 (9.90)
2	17.7	E(4β→6)E(4β→8)E (trimer III)	2.00	0.04 (5.77)	0.03 (3.20)
3	19.4	E(4β→8)E **B-2**	6.55	0.14 (10.28)	0.10 (11.30)
4	22.2	Unknown tetramer	3.32	0.07 (9.29)	0.04 (7.66)
5	30.2	E(4β→8)E(4β→8)E **C-1**	10.58	0.24 (7.03)	0.16 (6.02)
6	32.8	E(4β→8)E(4β→8)E(4β→8)E **D-1**	15.91	0.35 (5.14)	0.28 (5.92)
7	34.8	Unknown tetramer	2.78	0.05 (5.59)	0.04 (3.82)
8	35.8	Unknown procyanidin	2.38	0.04[b]	0.01[b]
9	37.0	Unknown procyanidin	1.77	0.03[b]	0.01[b]
10	38.5	E(4β→8)E(4β→8)E(4β→8)E(4β→8)E **E-1**	10.42	0.23 (8.09)	0.23 (8.58)
11	40.2	Unknown procyanidin	1.12	0.02[b]	–
12	42.7	Hexamer **F**	6.58	0.12 (8.33)	0.13 (15.94)
13	45.9	Unknown procyanidin	3.93	0.08[b]	0.02[b]
14	48.2	Unknown procyanidin	1.95	0.04[b]	0.01[b]
15	50.2	E(4β→6)E **B-5**	2.36	0.05 (6.80)	0.04 (5.68)
16	53.0	Unknown procyanidin	1.50	0.02[b]	0.01[b]
17	55.3	E(4β→8)E(4β→6)E (trimer II)	1.20	0.03 (9.41)	0.03 (18.62)
				1.58% (RSD 7.7%)	**1.15%** (RSD 8.8%)

C, (+)-catechin; E, (−)-epicatechin; d.w., dry mass.
[a] Concentrations of OPs in Fig. 2. corresponding to 5 mg of leaves/10 μl.
[b] Calculated as pentamer; RSD% in parentheses.

3.4. Quantitation

The quantitative determination of OPs was performed using the isolated main OPs as external standards. Concentrations of all the dimers were calculated using procyanidin **B-2** as external standard, trimers by **C-1**, tetramers by **D-1**, the pentamer and unidentified procyanidins by **E-1**, and the hexamer by **F**. The OPs were determined specifically due to fractionation of the phenolic compounds during sample preparation. The leaves contained 1.6% of OPs (dry mass), including 0.2% of unidentified OPs, i.e., peaks 8, 9, 11, 13, 14 and 16 (in Table 1). The flowers contained 1.2% of OPs, including 0.1% of unidentified OPs, i.e., peaks 8, 9, 13, 14, and 16 (in Table 1).

The unidentified peaks on the chromatograms were assumed to be isomeric pentameric and hexa-

Table 2
Calibration data for oligomeric procyanidins

Oligomeric procyanidin	Linear range (mg/ml)	Calibration curves	r[a]	Limit of quantitation, LOQ[b] (mg/ml)
Dimer **B-2**	0.05–4.00	$y=0.0016+0.0076x$	0.9999	0.05
Trimer **C-1**	0.05–4.00	$y=-0.0026+0.0068x$	0.9997	0.05
Tetramer **D-1**	0.09–2.64	$y=0.0023+0.0030x$	0.9968	0.09
Pentamer **E-1**	0.07–2.70	$y=-0.0007+0.0028x$	0.9999	0.07
Hexamer[c] **F**	0.12–0.81	$y=0.0005+0.0013x$	0.9978	0.12

[a] Correlation coefficients of equation $y = a + bx$, where x is procyandin concentration and y peak area.
[b] LOQ was determined with a 10-μl loop.
[c] Hexamer *F* was not pure, and the quantity of hexamer in the samples was calculated as the peak area (%) out of the total peak areas.

meric procyanidins on the basis of their UV spectra and R_f values, and they were calculated as pentamers. The dried fruits contained 0.2% of OPs (in Table 3). The proportion of OPs in the dried fruits was only approximately 10% of that of the OPs measured in the leaves. The OP pattern in the flowers, leaves and fruits was similar, although their total concentrations varied, depending on the part of the plant.

The concentrations of procyanidin dimers in *Crataegus* leaves and flowers are close to the values obtained by Rohr et al. [10], but the concentration of trimer **C-1** is considerably lower in their plant samples (0.06%). Vanhaelen and Vanhaelen-Fastre [19] carried out TLC–densitometric determination of dimer **B-2** and trimer **C-1**, and another unknown trimer, in flowers and leaves of *C. laevigata* using vanillin reagent and (−)-epicatechin as the standard. In their study, the concentration of **B-2** varied from 0.87 to 1.09%, and that of the trimers from 0.79 to 1.02% of the dry mass. The concentrations of dimers and trimers were higher than those obtained in this study. The proportion of procyanidins is also influenced by the date and site of harvesting [20,21]. The content of OPs was the highest at blossom time in the leaves of *C. monogyna* [20]. The procyanidin concentration in fruits is dependent on the degree of maturity of the fruits, the concentration being the highest in immature fruits [20]. The concentrations of minor procyanidins are not absolute, because they were not available in sufficient quantities to make standard curves. The use of (−)-epicatechin as a standard substance in the quantitative determination of OPs is not completely correct.

Table 3
Concentrations of oligomeric procyanidins in the leaves, flowers and fruits of *C. laevigata* (% of dry mass) and mean RSD%

Oligomeric procyanidins	% Dry mass		
	Leaves	Flowers	Fruits
Dimers	0.22	0.15	0.06
Trimers	0.31	0.22	0.06
Tetramers	0.47	0.36	0.02
Pentamer	0.23	0.23	0.01
Hexamer	0.12	0.13	n.d.
Unknown OPs	0.23	0.06	n.d.
Total (%)	**1.58**	**1.15**	**0.15**
Mean RSD (%)	7.7	8.8	12.3

d.w., dry mass; n.d., not detected.

Only a few OPs, mainly dimers and trimers, have been analysed quantitatively in other plants [13,14,18]. For example, dimeric procyanidins and trimer **C-1** have been determined from grape seeds by reversed-phase HPLC, and the concentrations of dimers varied between 0.03–0.25% [13] and 0.02–0.37% [14]. The present result of 0.06–0.22% of dimers in the fruits, flowers and leaves of hawthorn (Table 3) comes close to the concentrations of dimers in grape seeds.

4. Conclusions

The present HPLC method can be used for the qualitative and quantitative analysis of a complex series of oligomeric procyanidins of hawthorn up to the hexameric level. The compounds included procyanidin dimers epicatechin-(4β→8)-epicatechin (**B-2**), catechin-(4α→8)-epicatechin (**B-4**), epicatechin-(4β→6)-epicatechin (**B-5**), procyanidin trimers epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (**C-1**), epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin (trimer II), epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin (trimer III), a tetramer (**D-1**) and a pentamer (**E-1**) both consisting of (−)-epicatechin units linked through **C-4**β/**C-8** bonds. Concentrations of two unknown tetramers and a hexamer (**F**) were also quantified. This study demonstrates that it is possible to determine the concentrations of individual larger procyanidins in hawthorn, such as tetramers, pentamers and a hexamer. To our knowledge, there are no earlier reports of their quantitative results for *Crataegus* sp. The pattern of oligomeric procyanidins in the leaves, flowers and fruits was similar, but the concentrations varied. The method was valid with respect to repeatability, recovery, linearity, and sensitivity. The OPs were specifically determined due to fractionation of the phenolic compounds during sample preparation.

References

[1] H.P.T. Ammon, R. Kaul, Dtsch. Apoth. Z. 134 (1994) 2521.

[2] H.P.T. Ammon, R. Kaul, Dtsch. Apoth. Z. 134 (1994) 2631.
[3] A. Müller, W. Linke, W. Klaus, Planta Med. 65 (1999) 335.
[4] ESCOP, European Scientific Cooperative for Phytotherapy. Proposal for a European Monograph on the Medicinal Use of *Crataegus*. Vol. 2 (1992).
[5] E. Haslam, in: Practical Polyphenolics from Structure to Molecular Recognition and Physiological Action, Cambridge University Press, New York, 1998.
[6] G.E. Rohr, G. Riggio, B. Meier, O. Sticher, Phytochem. Anal. 11 (2000) 113.
[7] J.F. Hammerstone, S.A. Lazarus, H.H. Schmitz, J. Nutr. 130 (2000) 2086S.
[8] A. Hiermann, Th. Kartnig, S. Azzam, Sci. Pharm. 54 (1986) 331.
[9] J. Hölzl, A. Strauch, Planta Med. 32 (1977) 141.
[10] G.E. Rohr, B. Meier, O. Sticher, J. Chromatogr. A 835 (1999) 59.
[11] A. Rehwald, B. Meier, O. Sticher, J. Chromatogr. A 677 (1994) 25.
[12] D. Treutter, C. Santos-Buelga, M. Gutmann, H. Kolodziej, J. Chromatogr. A 667 (1994) 290.
[13] V. Katalinic, J. Chromatogr. A 775 (1997) 359.
[14] T. Fuleki, J.M. Ricardo da Silva, J. Agric. Food Chem. 45 (1997) 1156.
[15] S.A. Lazarus, G.E. Adamson, J.F. Hammerstone, H.H. Schmitz, J. Agric. Food Chem. 47 (1999) 3693.
[16] R.L. Prior, S.A. Lazarus, G. Cao, H. Muccitelli, J.F. Hammerstone, J. Agric. Food Chem. 49 (2001) 1270.
[17] U. Svedström, Dissertation, University of Helsinki, Helsinki, 2000.
[18] S. de Pascual-Teresa, C. Santos-Buelga, J.C. Rivas-Gonzalo, J. Agric. Food Chem. 48 (2000) 5331.
[19] M. Vanhaelen, R. Vanhaelen-Fastre, J. Pharm. Biomed. Anal. 7 (1989) 1871.
[20] Th. Kartnig, A. Hiermann, S. Azzam, Sci. Pharm. 55 (1987) 95.
[21] Th. Kartnig, A. Gruber, Sci. Pharm. 56 (1988) 271.