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Determination of hamamelitannin, catechins and gallic acid in witch hazel bark, twig and leaf by HPLC

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Abstract

An HPLC method for the determination of hamamelitannin, catechins and gallic acid in witch hazel bark, twig and leaf has been developed. The separation system consisted of a C18 reversed-phase column, a gradient elution system of methanol/water and orthophosphoric acid, and a photodiode array detector. The concentrations of hamamelitannin, gallic acid, (+)-gallocatechin and (+)-catechin were 4.77, 0.59, 0.22 and 0.39% (w/w), respectively, in the bark. Hamamelitannin and catechins were also detected in the leaves at concentration less than 0.04% (w/w). This method is simple, sensitive and reproducible, ideally suited for rapid, routine analysis.

Keywords: Witch hazel; Hamamelitannin; Catechins; Gallic acid; HPLC

1. Introduction

Witch hazel (*Hamamelis virginiana* L.) is a deciduous shrub or small tree native to damp woods in eastern North America and Canada. Extracts and distillates from witch hazel bark, twigs and leaves are widely used as components of skin care products and in dermatological treatment of sun burn, irritated skin, atopic eczema [1] and to promote wound healing via anti-inflammatory effects [2]. Hamamelitannin (2′,5-di-*O*-galloyl-hamamelose) is a main component of bark extract of witch hazel. Recently, Habtemariam [3] reported that 1–100 μM of hamamelitannin inhibited the tumour necrosis factor α-mediated endothelial cell death and DNA fragmentation in a dose-dependent manner; and the protective effect of hamamelitannin was comparable to that of a related compound, (−)-epigallocatechin gallate, which is widely regarded as the major antioxidant in green tea extract [4,5]. Hamamelitannin, at a minimum concentration of 50 μM, was also found to have a high protective activity against cell damage induced by peroxides [6].

Despite many reports on the medical properties of witch hazel preparations [7–9], only a limited number of papers have been published on the determination of chemical compounds in these materials [10]. Furthermore, no report on the

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determination of hamamelitannin has been found to the date.

This paper presents an HPLC method for the determination of hamamelitannin, catechins and gallic acid in witch hazel materials.

2. Material and methods

2.1. Materials

Various samples of dried witch hazel bark, twigs and leaves were supplied by Botanical & Natural Products Ltd (Hants, England).

2.2. Reagents and chemicals

Methanol (HPLC), ethanol (AR grade), acetone (AR grade) and orthophosphoric acid (AR grade) were purchased from Fisher Scientific (Essex, UK). Hamamelitannin standard was purchased from Extrasynthese (France). Standards of (+)-gallocatechin [(+)-GC], (−)-epigallocatechin [(−)-EGC], (+)-catechin [(+)-C], and gallic acid were purchased from Sigma Chemical Co. (Dorset, UK). The water used in HPLC and for sample preparation was produced with a Super Purity Water System (Purite Ltd, England) with a resistivity over 17.5 MΩ cm.

2.3. Preparation of standard solution

Stock standard solutions were prepared separately by accurately weighing 10 mg of gallic acid, (+)-GC, (−)-EGC, (+)-C and hamamelitannin reference standards into a 10-ml volumetric flask and dissolving in water with the aid of sonication. Working standard solutions, 0.3–100 μg/ml, were prepared by dilution with water from the stock standard solutions.

2.4. Sample preparation

All witch hazel samples were ground to powder. About 100 mg bark, 250 mg twigs, or 1000 mg leaves, were each accurately weighed into a 30-ml tube and extracted with 25 ml water with the aid of sonication for 2 min. The solution was centrifuged at 4500 rpm for 5 min, the supernatant transferred to a 50-ml volumetric flask and the solid residue re-extracted using 20 ml water with sonication and centrifugation as above. The supernatants were combined and made up to 50 ml with water. All samples were centrifuged at 13 000 rpm for 10 min prior to HPLC analysis.

2.5. Instrumentation

An HP 1100 series liquid chromatograph system comprising vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment and diode array detector was used. The column used was a Kingsorb 5μ C18, (150 × 4.6 mm). The column was maintained at 30 °C. Solvents used for separation were 0.1% (v/v) orthophosphoric acid in water (eluent A) and 0.1% (v/v) orthophosphoric acid in methanol (eluent B). The gradient used was: 0–15 min, 21% B; 15–20 min, linear gradient from 21 to 50% B. The flow rate was 1.0 ml/min and detection wavelength was 210 nm. Sample injection volume was 10 μl. The chromatographic peaks of the analytes were confirmed by comparing their retention times and UV spectra with those of the reference standards. Working standard solutions were injected into the HPLC, and peak area responses obtained. Standard graphs were prepared by plotting concentration versus area. Quantification was carried out from integrated peak areas of the samples using the corresponding standard graph.

3. Results and discussion

3.1. Separation of hamamelitannin, catechins and gallic acid

Several mobile phases, including methanol–water and acetonitrile–water in combination with acetic acid or phosphoric acid, were tested. Eventually, it was found that a water–methanol system with phosphoric acid, as described in Section 2.5, gave the best separation of hamamelitannin, catechins and gallic acid. Fig. 1a demonstrates the separation

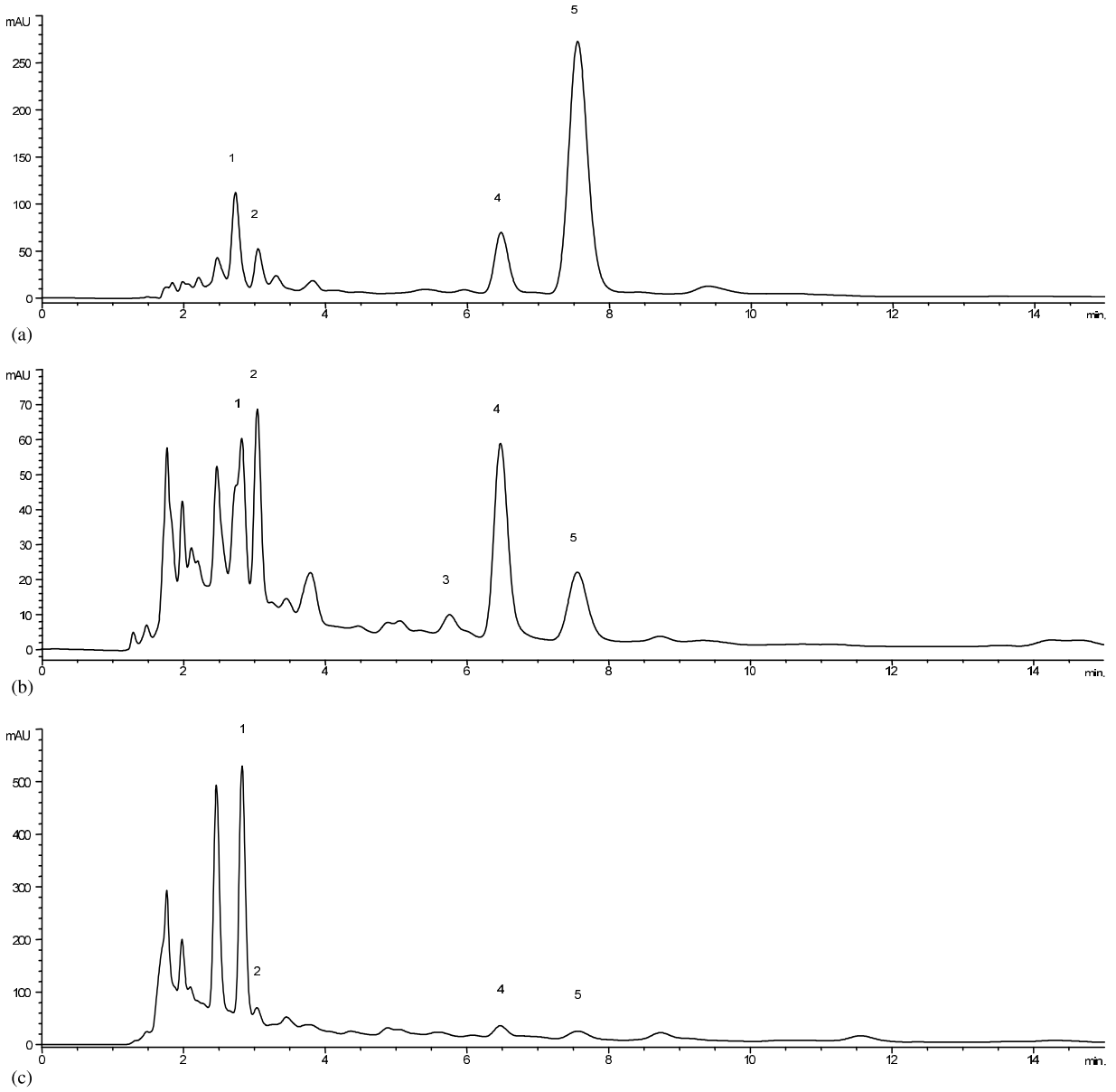

Fig. 1. Chromatograms of witch hazel samples, (a) bark; (b) twigs; (c) leaves. Peak identification: 1: gallic acid; 2: (+)-GC; 3: (−)-EGC; 4: (+)-C; 5: hamamelitannin.

obtained for a typical sample of witch hazel bark. It can be seen from this that a good separation can be achieved within 15 min using the conditions described. The remainder of the gradient conditions ensures efficient column washing.

3.2. Comparison of different solvents for the extraction of hamamelitannin, catechins and gallic acid

Ethanol, acetone, and acetonitrile, all at 15% (v/v) in water, were used to investigate the effect of

solvents on the extraction of hamamelitannin and catechins from witch hazel bark. The results were compared to those obtained with water as the extraction solvent. It was found that there was little difference using 15% ethanol, 15% acetonitrile or water, although with 15% acetone the extraction was slightly lower. As it is usual to prepare extracts from witch hazel using various concentrations of ethanol as extraction solvent, the effect of ethanol content on extraction was examined. It was found that good separation between gallic acid and (+)-GC could not be achieved if the sample was prepared with concentration of ethanol higher than 15%. As can be seen in Fig. 2, when 60% ethanol was used, the chromatography deteriorated due to injection solvent effects: the injection solvent was stronger than the mobile phase. However, once the ethanol concentration in the extract was diluted with water to less than 15% (v/v) prior to HPLC analysis, good separation could still be achieved. This demonstrates that when analysing ethanol extracts of witch hazel, it is important to ensure that the concentration of ethanol in the final sample solution for HPLC analysis is less than 15%. The same phenomenon was found in the analysis of tea catechins [11]. Based on the above observations, water is regarded as the best solvent for sample preparation.

The effect of extraction time on the content of hamamelitannin, catechins and gallic acid was investigated using water as the solvent. It was found that only 2 min of sonication or shaking was sufficient to extract all the analytes.

3.3. Stability of the analytes during analysis

The stability of hamamelitannin, catechins and gallic acid in extracted solutions was investigated. As a result, at room temperature, all of the analytes were stable for about 6 h and, after 8 h, a decrease of 3.8, 19.8, 3.0, and 2.6% for (+)-GC, (−)-EGC, (+)-C and hamamelitannin, respectively, and an increase of 10.1% for gallic acid were observed. This instability of (−)-EGC was also observed in green tea infusions [12]. Table 3 shows the precision of the analysis both within a day (<8 h) and between days (24–36 h) under the chromatographic conditions described above. It can be seen that for analyses performed within a day the coefficients of variation for all analytes were satisfactorily low, except (−)-EGC; for analyses performed between days, (−)-C and hamamelitannin retained reasonable coefficients of variation, while these for gallic acid, (+)-GC

Table 1
Characteristics of the calibration curves

Compound	Linear range (μg/ml)	R^2	LOL (%)
GA	0.3–60.7	0.9993	99.1
(+)-GC	0.5–68.7	1.0000	99.8
(−)-EGC	0.5–100.0	1.0000	99.8
(+)-C	0.5–68.2	1.0000	99.9
Hamamelitannin	0.3–100.0	0.9997	98.9

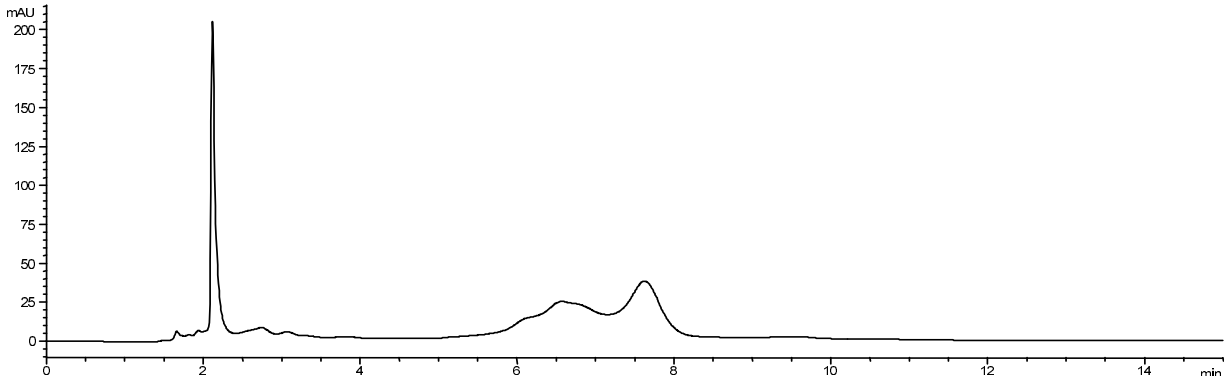

Fig. 2. Chromatogram of a sample prepared with 60% (v/v) ethanol.

Table 2
Performance characteristics

Compound	AS (μg/ml)	LOD_{approx} (μg/ml)	LOQ_{approx} (μg/ml)	Recovery (%)
GA	0.15	0.43	1.52	92.8
(+)-GC	0.16	0.46	1.53	91.8
(−)-EGC	0.20	0.58	1.92	89.6
(+)-C	0.08	0.22	0.72	97.0
Hamamelitannin	0.94	2.56	9.36	107.0

Table 3
Precision (coefficient of variations) of the detection (R.S.D.%)

Compound	Within a day	Between days
GA	1.72	10.32
(+)-GC	2.59	8.83
(−)-EGC	7.50	18.82
(+)-C	1.06	3.35
Hamamelitannin	0.15	3.73

and (−)-EGC deteriorated significantly. However, it was found that if sample solutions were kept in a freezer at about −10 °C for 1 week the decrease of all analytes was less than 2%.

3.4. Validation of the method

Calibration graphs for hamamelitannin, catechins and gallic acid were constructed using seven levels of concentration which covered the concentration ranges expected in the various samples. The characteristics of the calibration curves, including the range of linearity, the square of correlation coefficient (R^2) and on-line linearity (LOL) for each analyte are given in Table 1. LOL is determined by the following equation [13,14]:

$$\text{LOL}\ (\%) = 100 - \text{R.S.D.}(b)$$

where R.S.D.(b) is the relative standard deviation of the slope (expressed as a percentage). It can be seen that an excellent linearity was observed for all analytes over the range studied both in the terms of the correlation coefficients ($R^2 > 0.9989$) and LOL (>98%).

According to an ALAMIN program [13], analytical sensitivity (AS) is determined by the ratio of S_s/b, in which S_s is the residual standard deviation and b is the slope of the calibration curve. The limit of detection (LOD_{approx}) is determined by the following equation:

$$\text{LOD}_{approx} = 3(S_s/b)[(n-2)/(n-1)]^{1/2}$$

where n is number of total measurements for each calibration set. The limit of quantitation (LOQ_{approx}) is calculated by replacing 3 with 10 in the above equation. The results are shown in Table 2. It can be seen from the table that the limits are low enough to determine all analytes in the witch hazel samples.

The selectivity of this method and the efficiency of the column are evaluated by the resolution (Rs) of two vicinal peaks in the case of a bark sample. It was found that the minimum Rs was >2.3 between gallic acid and (+)-GC, and maximum Rs was 4.1 between (−)-C and hamamelitannin.

The recovery was determined by spiking a sample with three different additions of gallic acid, (+)-GC, (−)-EGC, (+)-C and hamamelitannin standard solutions, respectively. The recovery was found to be 89.6–107.0% (Table 2).

Running a blank injection after the analysis of either standard or witch hazel sample solutions showed no memory effect.

3.5. Quantitative measurement of different samples

Different parts of witch hazel, including the leaves, twigs and bark are used in a variety of applications. Fig. 1a–c show the chromatograms of witch hazel bark, twigs and leaves, respectively, and Table 4 shows their content of hamamelitannin, catechins and gallic acid. As can be seen, bark had the highest content of these analytes except (−)-EGC. The content of hamamelitannin in the

Table 4
Content of hamamelitannin, catechins and gallic acid (% w/w)

Compound	Bark	Twigs	Leaves
Gallic acid	0.59	0.12[a]	0.21
(+)-GC	0.22	0.12	0.03
(−)-EGC	n.d.	0.03	n.d.
(+)-C	0.39	0.16	0.02
Hamamelitannin	4.77	0.18	0.04

n.d., not detected.
[a] Including an unknown compound.

twigs was undoubtedly due to its presence in the thin bark on the outside of these twigs. In the twig sample, the content of gallic acid could not be accurately quantified due to its co-elution with an unknown compound. Among the witch hazel samples detected, only (−)-EGC was detected in low content in twigs. In witch hazel leaves, gallic acid was present at about 0.25% (w/w), but the contents of hamamelitannin and catechins were very low. This was contrary to a record of 8% hamamelitannin in leaves of witch hazel in Dr Duke's Phytochemical and Ethnobotanical Databases [15], in which the method of detection was not mentioned. It is possible that the data was derived by spectrophotometric measurement and other substances or other forms of tannin were included.

4. Conclusions

This method is simple and sensitive, and the limits of detection and quantitation are low enough to analyse hamamelitannin, (+)-GC, (−)-EGC, (+)-C and gallic acid in witch hazel plant materials. Therefore, the method is ideally suited for rapid, routine analysis.

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