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Determination of the major constituents in fruit of *Arctium lappa* L. by matrix solid-phase dispersion extraction coupled with HPLC separation and fluorescence detection

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ABSTRACT

The arctiin and arctigenin in the fruit of *Arctium lappa* L. were extracted by matrix solid-phase dispersion (MSPD) and determined by high-performance liquid chromatography (HPLC) with fluorescence detection. The experimental conditions for the MSPD were optimized. Silica gel was selected as dispersion adsorbent and methanol as elution solvent. The calibration curve showed good relationship ($r > 0.9998$) in the concentration range of 0.010–5.0 μg mL^{-1} for arctiin and 0.025–7.5 μg mL^{-1} for arctigenin. The recoveries were between 74.4% and 100%. The proposed method consumed less sample, time and solvent compared with conventional methods, including ultrasonic and Soxhlet extraction.

1. Introduction

The dried fruit of *Arctium lappa* L. is one of the most popular traditional Chinese medicinal materials [1]. Arctiin and arctigenin are major constituents in the fruit of *A. lappa* L. [2–6]. These constituents have been reported to show a variety of biological activities and a number of important pharmacological properties, such as the antagonistic effect on the PAF receptor [7], cytotoxic, anti-proliferative [8,9], calcium antagonist [3] and anti-carcinogenesis [10] activities. Furthermore, arctigenin was found to inhibit strongly the replication of human immunodeficiency virus type 1 (HIV-1; strain HTLY-III B) in vitro and the reverse transcriptase activity of HIV-1 [11].

Arctiin and arctigenin are well known as the beneficial components and have rigid planar structure possessing fluorescent chromophores. In the process of chemical evaluation or standardization of *A. lappa* L. and its products, the inherent arctiin and arctigenin were chosen as "marker compounds". In the literature few studies on HPLC determination of arctiin and arctigenin in *A. lappa* L. were reported. Lü et al. [12] reported a microemulsion electrokinetic chromatography to separate arctiin and arctigenin in the fruit of *A. lappa* L. Liu et al. [6] employed single quadrupole mass spectrometry to characterize lignans in *A. lappa* L. and reported the isolation and identification of arctiin in the leaves of *A. lappa* L. Lou et al. [13] reported the HPLC coupled with mass spectrometry detection for identification of phenolics (including arctiin) in the leaves of *A. lappa* L.

The extraction of active compounds from medicinal plants has been traditionally performed using solvent extraction or maceration extraction. The traditional extraction methods are time consuming, labor intensive, and require large amounts of solvent and sample although they are often effective. Following the rapid development of analytical techniques, the trends in the analytical extractions have been a movement toward less (organic) solvent consumption and faster extraction time. Matrix solid-phase dispersion (MSPD) is a simple and cheap sample preparation procedure involving simultaneous disruption of various solid and semi-solid materials and extraction of target compounds from the materials. This method has been applied widely to the extraction of herbicides, pesticides and other pollutants in fruits, vegetables and plant material [14–17]. However, only a few reports using MSPD technique to extract constituents in medicinal plants were published [18–20]. It permits complete fractionation of the sample matrix components and has the ability to selectively isolate a single compound or several kinds of compounds from the sample. The performance of MSPD is mainly affected by the column pack-

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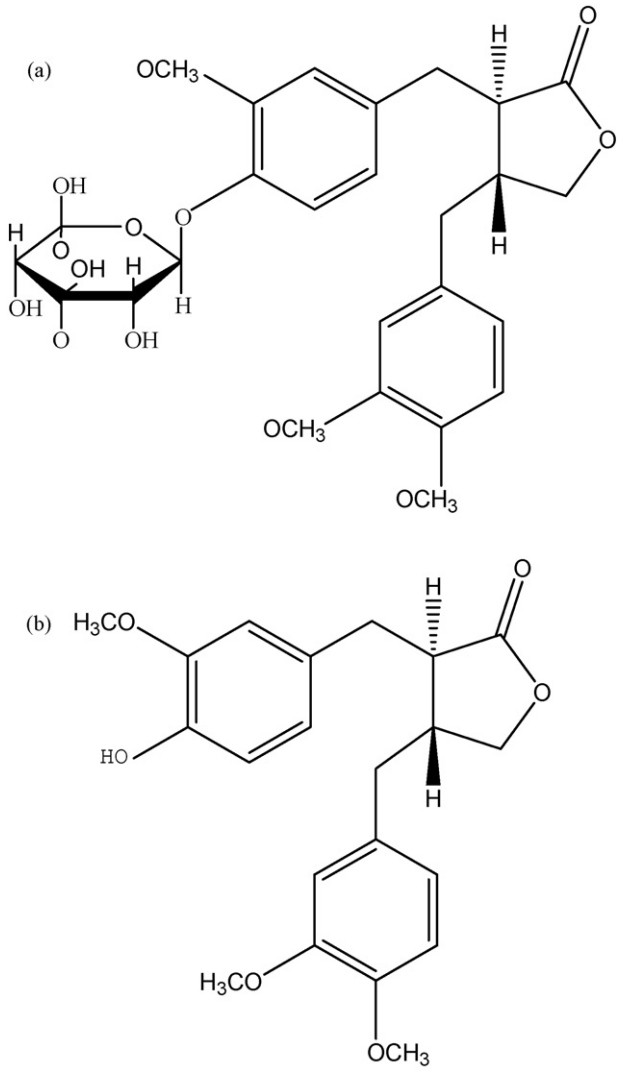

Fig. 1. Chemical structures of arctiin (a) and arctigenin (b).

ing technique and the elution procedure. In MSPD, the analyzed sample is blended with a suitable dispersion adsorbent to form a homogenous packing material. Then, the blended material is transferred and packed into a column. The analytes are eluted with a relatively small volume of suitable solvent while interfering matrix compounds are selectively retained on the column. Compared with classical methods, the solvent amount consumed is less and extraction time generally shorter [21,22].

In this work, MSPD coupled with HPLC-fluorescence detection was first applied for the extraction and determination of arctiin and arctigenin from the fruit of *A. lappa* L.

2. Experimental

2.1. Materials and chemicals

The standard arctiin (purity ≥97%) was obtained from China Drug Biological Product Qualifying Institute (Beijing, China) and arctigenin (purity ≥98%) was from BBT Inc. (Tianjing, China). The chemical structures of the compounds are shown in Fig. 1. HPLC grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Analytical grade methanol, acetone, ethyl acetate and light petroleum were purchased from Beijing Chemical Factory (Beijing, China). Water was purified with a distilling apparatus (Ronghua company, Jiangsu, China) and filtered through a 0.45 μm membrane.

Silica gel (200–300 mesh), C18 (60–80 mesh) and neutral alumina (200 mesh) were obtained from Chinese Medical and Biological Products Institute (Beijing, China). Before use, neutral alumina was baked at 650 °C for 4 h and dried at 100 °C for 2 h; silica gel was baked at 130 °C for 2 h; C18 was sequentially washed with *n*-hexane, dichloromethane and methanol and dried naturally, and then, the materials were stored in the desiccator for future use.

Four kinds of *A. lappa* L. samples (named as samples 1–4) cultivated in different areas were bought from local drugstores. In the study, all experiments were performed on sample 1 except for the experiments mentioned in Section 3.4. The fruit of *A. lappa* L. was dried thoroughly in the cabinet drier at 40 °C for 12 h. The samples were powdered and passed through a 60 mesh sieve.

2.2. Preparation of standards

For each analyte, the standard stock solution was prepared by dissolving it in methanol to obtain final concentration of 270 μg mL^{-1}. The arctiin and arctigenin standard stock solutions were stored at 4 °C. Working solutions were prepared by diluting the stock solutions with the mobile phase.

2.3. Determination by HPLC

HPLC determination was carried out on a Shimadzu RF-10AXL HPLC system (Shimadzu, Kyoto, Japan) with a fluorescence detector. The fluorescence spectra of arctiin and arctigenin were measured on a Shimadzu RF-5301PC fluorescence spectrophotometer (Kyoto, Japan). The chromatographic separation of the analytes was achieved with a C18 column (150 mm × 4.6 mm i.d., 5 μm, Agela Technologies, USA) operating at 30 °C. The mobile phase consisted of water (A) and acetonitrile (B). Gradient program was as follows: 0–7 min, 25–46% B; 7–11 min, 46–10% B; 11–15 min, 10% B. The flow rate of the mobile phase was maintained at 1 mL min^{-1}. The injection volume of sample solution was 20 μL.

2.4. MSPD extraction

0.050 g of sample and 0.15 g of dispersion adsorbent were placed in the agate mortar. The sample and the dispersion adsorbent were blended using a pestle. Once completely dispersed, the homogeneous mixture was transferred into a column with a layer of absorbent cotton on the bottom of the column. After transfer, a thin layer of absorbent cotton was added at the top of the sample mixture. And then the column was eluted with 7 mL of methanol. The target analytes were eluted out and collected in a 10 mL of volumetric flask. The collected eluate was filtered through a 0.45 μm membrane and used as the sample solution.

2.5. Ultrasonic extraction

0.50 g of sample was put into a 50 mL flask, into which 45 mL of methanol was added. The flask was immersed in the water bath of an ultrasonic cleaner (KQ-100DE Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, China) and sonicated for 20 min. Then, the extract was diluted to 50 mL with methanol. The resulting extract was filtered sequentially with filter paper and 0.45 μm filter membrane. The resulting solution constituted the sample solution for HPLC analysis.

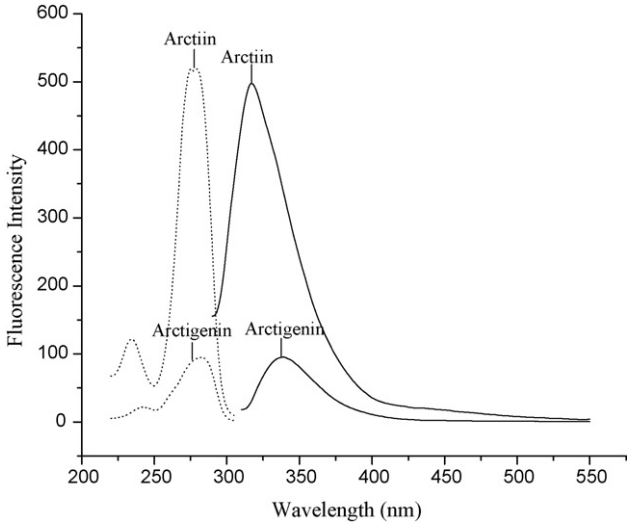

Fig. 2. The excitation (dotted line) and emission (solid line) spectra of arctiin and arctigenin.

2.6. Soxhlet extraction

2.0 g of sample and 40 mL of methanol were put into a Soxhlet distilling flask. The mixture was heated and refluxed for 16 h. The extract was transferred into a 50 mL of volumetric flask and diluted to the mark with methanol. After filtration with a 0.45 μm membrane filter, the resulting solution constituted the sample solution.

3. Results and discussion

3.1. HPLC performances

Fig. 2 shows the excitation and emission spectra of arctiin and arctigenin. The peak of excitation spectra at 280 nm in the wavelength range of 220–310 nm and the peak of emission spectra at 319 nm in the wavelength range of 290–500 nm are observed. Therefore, detection of arctiin and arctigenin was carried out using 280 and 319 nm as excitation and emission wavelength, respectively.

The mobile phase consisted of water and organic solvent. A good separation of analytes was achieved when acetonitrile was used as organic phase. So acetonitrile/water solution was used as the mobile phase. Fig. 3 presents the chromatograms of the standard solution and real samples. The retention time of arctiin and arctigenin are 6.4 and 11.5 min, respectively, and their resolution is satisfactory. The peak of arctigenin was very close to a small peak of impurity from the extract of sample. In order to separate the two peaks, the concentration of acetonitrile in mobile phase was reduced to 10%, which led the peak of arctigenin to be markedly asymmetric and wide.

3.2. Optimization of the MSPD procedure

Several dispersion adsorbents including, silica gel, neutral alumina and C18, were examined in order to find the most suitable dispersion adsorbent. The experimental results shown in Table 1 indicate similar results were obtained when the three kinds of dispersion adsorbents were used. However, silica gel was cheaper than other adsorbents. Thus, silica gel was selected as dispersion adsorbent. The effect of the mass ratio of silica gel to sample was examined. It is seen from Fig. 4 that the effect of the mass ratio on the yields of analytes is not significant. When the mass ratio is 3:1,

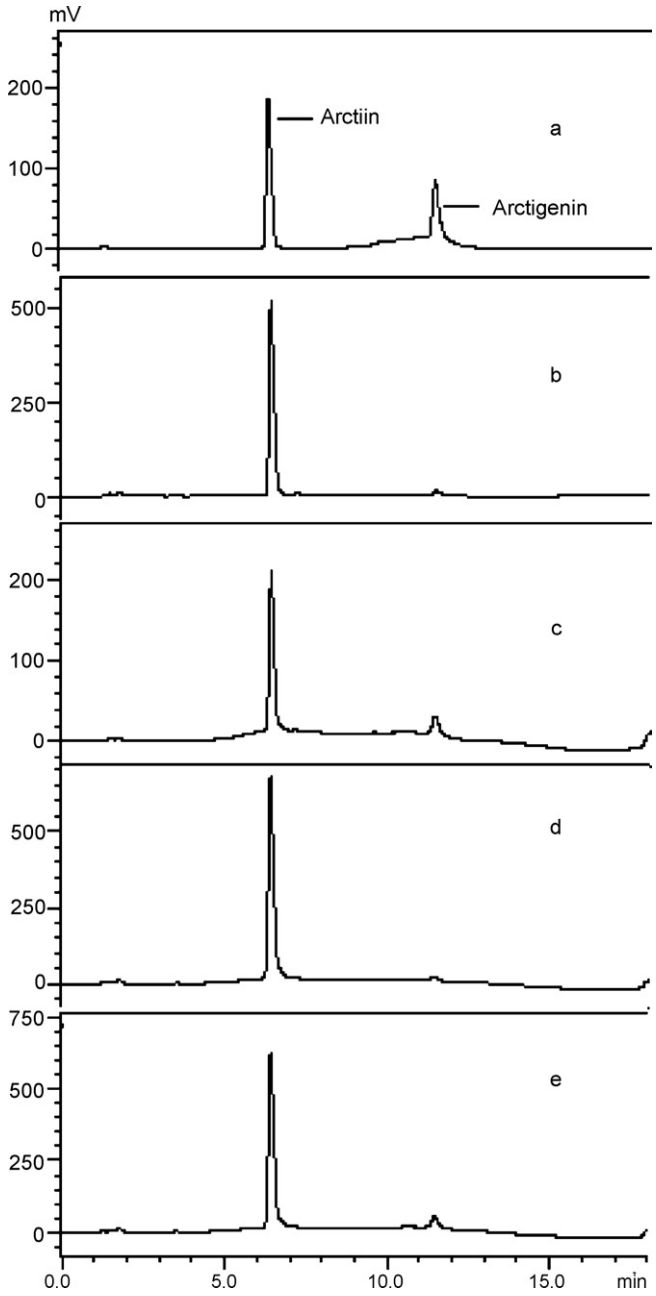

Fig. 3. Chromatograms of (a) the standard solution (concentration of both arctiin and arctigenin was 1.0 μg mL^{-1}), (b) the extract of sample 1, (c) the extract of sample 2, (d) the extract of sample 3 and (e) the extract of sample 4.

the extraction yield is the highest and that was therefore the mass ratio selected in this work.

The effect of elution solvents was studied in order to obtain the highest extraction yields for the analytes. Methanol, acetone, ethyl

Table 1
Extraction yields obtained using different dispersion adsorbents.

Dispersion adsorbents	Analyte	Extraction yields (mg g^{-1})
Silica gel	Arctiin	55.0
	Arctigenin	2.80
Neutral alumina	Arctiin	53.2
	Arctigenin	2.58
C18	Arctiin	54.2
	Arctigenin	2.78

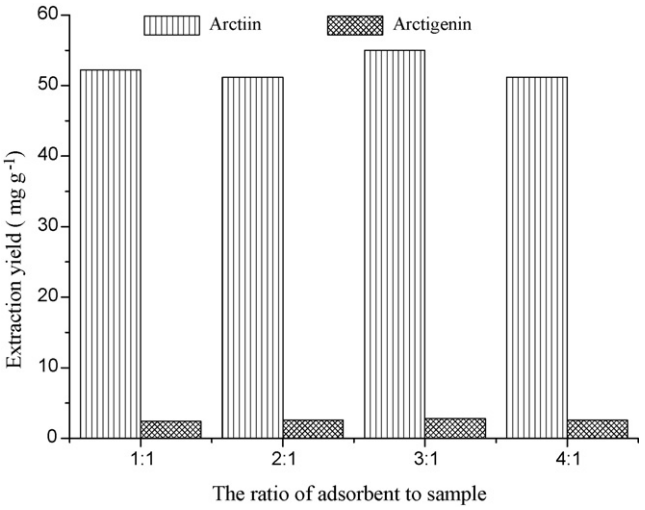

Fig. 4. The effect of the ratio of adsorbent to sample on extraction yields of arctiin and arctigenin. 7 mL of methanol was used as the elution solvent.

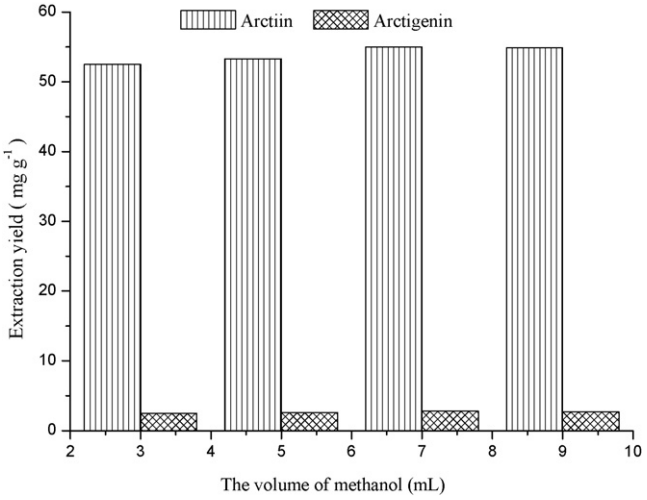

Fig. 6. The effect of volume of elution solvent on extraction yields.

acetate and light petroleum were tested because they were commonly used in MSPD. These solvents present dissimilar polarities. The stronger the polarity of the solvent, the stronger capability to elute the analytes (Fig. 5). Thus, methanol was used as elution solvent. The effect of volume of elution solvent on extraction yields of target compounds was investigated and the experimental results are shown in Fig. 6. The extraction yields of arctiin and arctigenin slightly increase when the solvent volume is from 3 to 7 mL. The extraction yields of arctiin and arctigenin are 55.0 and 2.80 mg g^{-1}, respectively, when the volume of elution solvent is 7 mL. No significant increase in extraction yields is observed when the volume of elution solvent is from 7 to 9 mL. So 7 mL of methanol was chosen as the elution solvent in further experiments.

3.3. Evaluation of the method

3.3.1. Linearity

The calibration curves were obtained by determining arctiin at six concentration levels (0.010, 0.050, 0.10, 0.50, 2.5 and 5.0 μg mL^{-1}) and arctigenin at six concentration levels (0.025, 0.25, 0.50, 1.0, 2.5, 5.0 and 7.5 μg mL^{-1}). The relationships between the analyte concentration (C) and measured peak area (A) were expressed as regression equations: $A = -9.6 \times 10^3 + 2.1 \times 10^6 C$ for arctiin and $A = -3.7 \times 10^4 + 1.2 \times 10^6 C$ for arctigenin, respectively. Good linearity were obtained in a range of 0.010–5.0 μg mL^{-1} with the correlation coefficient of 0.9999 for arctiin and 0.025–7.5 μg mL^{-1} with the correlation coefficient of 0.9998 for arctigenin.

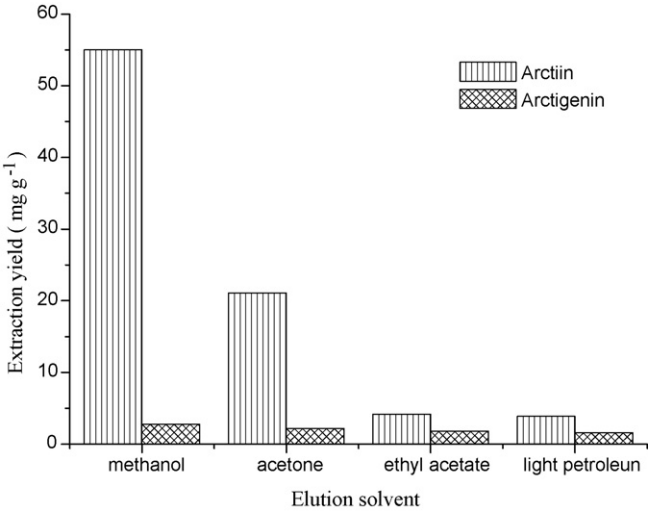

Fig. 5. The effect of elution solvents on the extraction yields.

3.3.2. Limits of detection

The limits of detection (LODs) were determined at the signal-to-noise ratios of 3. The LODs for arctiin and arctigenin were 0.0013 and 0.0042 μg mL^{-1}, respectively.

3.3.3. Repeatability

The repeatability of the MSPD procedure was assessed by evaluating the peak area variation of arctiin and arctigenin. Three replicates were performed. The relative standard deviations (RSDs) were between 1.9% and 2.4% for the intra-day and between 4.2% and 9.6% for inter-day assays. Therefore, the reproducibility of the MSPD procedure was acceptable.

3.3.4. Comparison of MSPD, ultrasonic and Soxhlet extraction

In order to evaluate the performances of MSPD, ultrasonic and Soxhlet extraction were also applied. The results are shown in Table 2. From these data, it can be seen that there are no significant differences in extraction yields for atrctiin, only in the case of arctigenin there is an apparent poorer yield for ultrasonic extraction compared with MSPD and Soxhlet. When Soxhlet extraction was applied, much more sample, time and solvent were consumed compared with MSPD and ultrasonic extraction. Considering the extraction yields and consumption of sample, time and solvent, MSPD extraction should be a comparatively better method.

Table 2
Comparison of MSPD, ultrasonic and Soxhlet extraction.

	MSPD	Ultrasonic	Soxhlet
Extraction yield of arctiin (mean ± SD[a], mg g^{-1})	55.0 ± 1.1	54.6 ± 1.1	57.6 ± 1.0
Extraction yield of arctigenin (mean ± SD, mg g^{-1})	2.80 ± 0.01	2.23 ± 0.01	2.84 ± 0.00
Sample (g)	0.05	0.5	2.0
Solvent (mL)	10	50	50
Time (h)	0.5	1.0	16.5

[a] Standard deviation (n = 3).

Table 3
The analytical results of samples.

Sample	Arctiin		Arctigenin	
	Extraction yield (mg g^{-1})	RSD (%, n = 3)	Extraction yield (mg g^{-1})	RSD (%, n = 3)
1	55.0	0.65	2.80	3.7
2	18.5	0.45	4.31	3.1
3	74.6	0.57	3.44	6.2
4	72.0	0.90	8.89	4.5

Table 4
Analytical results of the spiked samples.

Analyte	Original (mg g^{-1})	Added (mg g^{-1})	Found (mg g^{-1})	Recovery (%)	RSD (%, n = 3)
Arctiin	55.0	52.0	93.7	74.4	2.8
	55.0	26.0	78.3	89.6	4.2
Arctigenin	2.80	2.00	4.80	100	10
	2.80	1.00	3.52	90.0	11

3.4. Analysis of samples

To examine the applicability of the proposed method the samples obtained from four different cultivated areas were analyzed. The results (Table 3) indicate that the contents of arctiin and arctigenin are in the range of 18.5–74.6 and 2.80–8.89 mg g^{-1}, respectively. The differences in lignan concentrations in these samples are due to the difference in cultivated area, growth conditions and picking period.

Spiked samples were analyzed. The results are summarised in Table 4. The spiked samples were prepared by spiking the standard stock solutions into sample powders. To ensure the standard solution to be well distributed, a reasonable amount of methanol was added to moisten the sample powder and careful agitation was performed followed by an air-drying for 24 h at ambient temperature before sample analysis. The recoveries are from 74.4% to 100%. It can be seen that the RSD values for arctiin are less than 4.2%, which is quite suitable for quantification. For arctigenin, the RSD values are in the range of 10–11%. The relatively high RSD values might partly be due to tailed peak of arctigenin.

4. Conclusion

The method allows for an efficient and simultaneous determination of arctiin and arctigenin in the fruit of *A. lappa* L. and takes advantage of the fluorescence properties of the analytes. In addition, compared with traditional techniques the proposed extraction method consumes less sample, time and solvent.

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