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UNIVERSITY & RESEARCH

European Union Reference Laboratory

Memo

TO
Mr F. Verstraete
European Commission
Chairperson Working Group on Agricultural Contaminants in Food

FROM
Patrick Mulder

Dear Mr Verstraete,

On behalf of Dr. Patrick Mulder, I hereby send you an updated version of the results of a first brief survey on available validated analytical methods for individual hydroxyanthracene derivatives (HAD) present in botanical preparations of *Aloe*, *Rheum*, *Rhamnus* and *Cassia* species and the methods available to quantify the total HAD content in these products. The survey was carried out on June 30-July 6, 2020 with help of Dr. Aleksandrs Veršilovskis.

Please contact Dr Mulder or me if you have additional questions.

With kind regards,

Monique de Nijs

EURL mycotoxins & plant toxins
Wageningen Food Safety Research

Wageningen Food Safety Research

DATE
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SUBJECT
EURL-MP-report_002
Inventory analytical methods hydroxyanthracene derivatives

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Available validated analytical methods for the determination of aloe-emodin, emodin and danthron, and the methods available to quantify the total HAD content in botanical products.

Introduction

In 2018 the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) prepared an opinion on the safety of hydroxyanthracene derivatives (HAD) for use in food (EFSA 2018). Based on the data available, the Panel concluded that hydroxyanthracene derivatives should be regarded as genotoxic and carcinogenic unless there are specific data to the contrary, such as for rhein, and that there is a safety concern for extracts containing HADs although uncertainty persists.

Related to this opinion the Commission asked the EURL mycotoxins & plant toxins in food and feed to carry out a literature search for method(s) of analysis that can be widely used by official laboratories, which have been validated and proven to be able to reliably detect the following substances in botanical preparations:

1) aloe-emodin, emodin and danthron;
2) HADs/quantify the total HAD content in *Aloe* extracts/products;
3) HADs/quantify the total HAD content in *Rheum* extracts/products, *Cassia* extracts/products, and *Rhamnus* extracts/products.

The issue of sampling is also briefly addressed.

Literature search:

a. Scientific literature was search for the following terms:
   Compounds:
   1. emodin and aloe-emodin (dihyroxyanthraquinone), danthron (synthetic dihyroxyanthraquinone);
   2. aloin A and aloin B (anthrone glycoside);
   3. other hydroxyanthracene derivatives (HAD), including glycosides, diglycosides, see appendix EFSA opinion.

   Plant materials / food supplements / botanical extracts:
   1. root and rhizome of *Rheum palmatum* L. and/or *Rheum officinale* Baillon and/or their hybrids;
   2. leaves or fruits of *Cassia senna* L. and/or *Cassia angustifolia* Vahl;
   3. bark of *Rhamnus frangula* L., bark of *Rhamnus purshianus* D.C.;
   4. leaves of *Aloe barbadensis* Miller and/or various *Aloe* species, mainly *Aloe ferox* Miller and its hybrids.

b. The methods available in the collected publications were analysed for:
   1. Principle of the method.
   2. Scope of the method.
   3. LOD/LOQ.

Results

a. Literature related to methods for analysis of aloe-emodin, emodin and danthron in food supplements.

More than 3000 references were detected using the search terms, of which about 100 were relevant. The most interesting methods are presented in Table 1 and 4. Most methods include emodin and/or aloe-emodin and other closely related dihydroxyanthracene derivatives. Emodin is sometimes included in LC-MS/MS multimethods with a broad scope covering mycotoxins and/or plant metabolites. Almost no analytical methods were found for determination of danthron itself or in combination with aloe-emodin or emodin or other relevant compounds. This is likely due to the fact that danthron is a synthetic HAD, not supposed to be present in the plant materials. Multi-toxin methods could be used as a good starting point for method development for the target compounds.

Extraction of the supplements is most often carried out using (aqueous) methanol or ethanol, sometimes acidified with hydrochloric acid or acetic acid. After extraction, the suspension is centrifuged, sometimes after sonification and filtered through a 0.2 or 0.45 µm filter.

In most methods HPLC is used to separate the compounds. For screening purposes, detection is carried out using UV or DAD. For identification and quantification, LC-MS techniques are used (LC-MS/MS, LC-Orbitrap-MS, DESI-HRMS) and sometimes GC-MS.

Limits of detection and quantification are typically in the ng/mL to µg/mL range for the final extracts. When available, the LOD/LOQ in the starting material is also given in Table 1. LODs/LOQs in the starting material typically are in the 0.1-50 µg/g range. LC-MS/MS methods are generally somewhat more sensitive than HPLC-UV methods, but this also depends on the type of purification applied. Ref 16 of Table 4 provides a representative LC-MS/MS method for the analysis of aloe-emodin, emodin, chrysophanol and rhein. LOQs for this method range from 0.63 µg/g for emodin to 1.2 µg/kg for aloe-emodin, 2.5 µg/g for rhein and 25 µg/g for chrysophanol.

Table 1. Overview of representative LODs/LOQs reported in the literature for emodin and related hydroxyanthracene derivatives in food supplements.

Analyte	LOD in final extract	LOQ in final extract	LOD in dry matrix	LOQ in dry matrix	Technique	Ref. (Table 4)
Aloe-emodin			0.54 µg/g	-	HPLC-UV/LC-MS	2
Aloe-emodin	0.23 µg/mL	0.92 µg/mL			HPLC-UV	3
Aloe-emodin	0.04 µg/mL	0.12 µg/mL			HPLC-UV	4
Aloe-emodin	18 ng/mL	45 ng/mL	4.5 µg/g	11 µg/g	HPLC-DAD/LC-MS	8
Aloe-emodin	0.42 µg/mL	1.34 µg/mL			SFC-DAD	10
Aloe-emodin	0.01 µg/mL	0.03 µg/mL			HPLC-UV/LC-MS	11
Aloe-emodin	10 ng/mL	20 ng/mL			HPLC-UV	12
Aloe-emodin	20 ng/mL	-			GC-MS	13
Aloe-emodin	0.28 ng/mL	1.2 ng/mL	0.28 µg/g	1.2 µg/g	LC-MS/MS	16
Aloe-emodin	0.45 ng/mL	1.51 ng/mL	0.14 µg/g	0.45 µg/g	LC-QTOF-MS	23
Emodin			0.23 µg/g	-	HPLC-UV/LC-MS	2
Emodin	0.28 µg/mL	0.83 µg/mL			HPLC-UV	3
Emodin	0.06 µg/mL	0.2 µg/mL			HPLC-UV	4
Emodin			0.03 µg/g	0.1 µg/g	LC-MS/MS	5
Emodin	16.7 ng/mL	20 ng/mL	4.2 µg/g	5 µg/g	HPLC-DAD/LC-MS	8
Emodin			0.2 µg/g	0.6 µg/g	LC-MS/MS	9
Emodin	0.42 µg/mL	1.34 µg/mL			SFC-DAD	10
Emodin	10 ng/mL	-			GC-MS	13
Emodin	0.12 µg/mL	0.32 µg/mL	4.8 µg/g	12.8 µg/g	HPLC-UV	14
Emodin	0.19 ng/mL	0.63 ng/mL	0.19 µg/g	0.63 µg/g	LC-MS/MS	16
Emodin	0.09 µg/mL	0.24 µg/mL	9 µg/g	24 µg/g	HPLC-UV	17
Emodin	2 ng/mL	6 ng/mL			LC-Orbitrap-MS	18
Emodin	0.02 µg/mL	0.06 µg/mL			LC-Q-TOF-MS	20
Emodin	0.62 ng/mL	2.08 ng/mL	0.19 µg/g	0.62 µg/g	LC-QTOF-MS	23
Emodin-8-O-β-D-glucoside	0.15 µg/mL	0.9 µg/mL			LC-Orbitrap-MS	19
Emodin-8-O-β-D-gluco-pyranoside, ω-hydroxy-emodin	0.02 µg/mL	0.06 µg/mL			LC-Q-TOF-MS	20
Chrysophanol	0.0075 µg/mL	0.025 µg/mL	7.5 µg/g	25 µg/g	LC-MS/MS	16
Chrysophanol	2.53 ng/mL	8.44 ng/mL	0.76 µg/g	2.53 µg/g	LC-QTOF-MS	23
Physcion	0.12 µg/mL	0.32 µg/mL			HPLC-UV	14
Physcion	0.01 µg/mL	0.033 µg/mL	10 µg/g	33 µg/g	LC-MS/MS	16
Rhein	33.3 ng/ml	100 ng/ml	8 µg/g	25 µg/g	HPLC-DAD/LC-MS	8
Rhein	0.01 µg/mL	0.02 µg/mL			HPLC-UV/LC-MS	11
Rhein	0.83 ng/mL	2.5 ng/mL	0.83 µg/g	2.5 µg/g	LC-MS/MS	16
Rhein	0.14 µg/mL	0.44 µg/mL	14 µg/g	44 µg/g	HPLC-UV	17
Rhein	1.24 ng/mL	4.12 ng/mL	0.38 µg/g	1.24 µg/g	LC-QTOF-MS	23

b. Literature related to analysis of HADs/quantify the total HAD content in Aloe extracts/products.

More than 250 references were found using the search terms. The most relevant methods are presented in Table 2 and 5. There are only a limited number of methods available for analysis of aloin A/B in food supplements. Practical methods that quantify the total HAD content in food supplements of *Aloe* species were not identified.

Extraction of the supplements is most often carried out using (aqueous) methanol or ethanol, sometimes acidified with acetic acid, or with PBS buffer.

Mostly HPLC-UV and LC-MS techniques are used to quantify aloin A/B and glycosides. Some methods use GC-MS or CE-FLD.

Limits of detection and quantification are typically in the ng/mL to µg/mL range for the final extracts. When available, the LOD/LOQ in the starting material is also given in Table 2. LODs/LOQs in the starting material typically are in the 0.1-1 µg/g range. Ref 7 from Table 5 provides a representative LC-UV method for the analysis of Aloin A and B. Reported LOQs for this method are 0.23 µg/mL for aloin A and 0.21 µg/mL for aloin B in Aloe extracts.

Table 2. Overview of representative LODs/LOQs reported in the literature for HADs in Aloe extracts/products.

Analyte	LOD in final extract	LOQ in final extract	LOD in dry matrix	LOQ in dry matrix	Technique	Ref. (Table 5)
Aloin	0.0053 µg/mL	0.0161 µg/mL			HPLC-UV	10
Aloin A			0.05 µg/g	0.1 µg/g	GC-MS	3
Aloin A	0.092 µg/mL	0.23 µg/mL			HPLC-UV	7
Aloin A	7.3 ng/mL	24.5 ng/mL			Cap-CE-FLD	12
Aloin A	0.54 µg/mL	-			HPLC-UV	6
Aloin B	0.087 µg/mL	0.21 µg/mL			HPLC-UV	7
Aloin B	7.5 ng/mL	24.9 ng/mL			Cap-CE-FLD	12
Aloin A/B	0.4 µg/mL	1.5 µg/mL			Nano-LC-UV/MS	5
Aloin A/B	2 µg/spot	-			TLC+HPLC-UV	4
Aloin A/B	10 ng/mL	20 ng/mL			HPLC-UV	9
Aloin A/B, aloeresin, hydroxyaloin, aloinoside A/B			0.15 µg/g	-	HPLC-UV	2
Total antranoids	-	0.815 mg/mL			bsqHSOC (NMR)	13

c. Literature related to analysis of HADs/quantify the total HAD content in Rheum Cassia or Rhamnus extracts/products.

Less than 50 scientific publications were detected using these search terms. After reviewing only a few methods are available. About 500 scientific papers were found for the analysis of dianthrone glycosides (sennosides). A small number of scientific papers were identified on the analysis of the anthrone glycosides: aloinoside, cascaroside, glucofrangulin and dianthrones: palmidin C. The most relevant methods were selected and are presented in Table 3 and 6.

Extraction of the supplements is most often carried out using (aqueous) methanol or ethanol. After extraction, the suspension is centrifuged, sometimes after sonification and filtered through a 0.45 µm filter. Many reports were found on isolation of the compounds from the plant themselves, aiming at high quantities. These are not directly relevant for the analysis at low concentrations.

Authors use HPLC to separate the compounds. For screening purposes, detection is carried out using UV. Identification and quantification is carried out using DAD and less frequently with LC-(HR)MS.

Limits of detection and quantification are typically in the ng/mL to µg/mL range for the final extracts. When available, the LOD/LOQ in the starting material is also given in Table 3. LODs/LOQs in the starting material typically are in the 0.5-30 µg/g range.

Table 3. Overview of representative LODs/LOQs reported in the literature for HADs in Rheum Cassia or Rhamnus extracts/products.

Analyte	LOD in final extract	LOQ in final extract	LOD in dry matrix	LOQ in dry matrix	Technique	Ref. (Table 6)
Sennoside A	0.2 µg/mL	-			HPLC-UV	1
Sennoside A	0.01 µg/mL	-	0.5 µg/g	-	HPLC-UV	5
Sennoside A	0.8 µg/mL	2.1 µg/mL			HPLC-UV	7
Sennoside A	0.07 µg/mL	0.24 µg/mL	8.8 µg/g	30 µg/g	HPLC-UV	13
Sennoside A	1.4 ng/mL	4.7 ng/mL	0.84 µg/g	2.8 µg/g	LC-TOF-MS	14
Sennoside A	1 µg/mL	2 µg/mL			HPLC-UV	15
Sennoside A	0.7 µg/mL	2.31 µg/mL			LC-MS	16
Sennoside B	0.1 µg/mL	-			HPLC-UV	1
Sennoside B	0.01 µg/mL	-	0.5 µg/g	-	HPLC-UV	5
Sennoside B	0.6 µg/mL	2 µg/mL			HPLC-UV	7
Sennoside B	0.05 µg/mL	0.2 µg/mL	6.25 µg/g	25 µg/g	LC-UV	13
Sennoside B	1.3 ng/mL	4.3 ng/mL	0.78 µg/g	2.6 µg/g	LC-TOF-MS	14
Sennoside B	2 µg/mL	3 µg/mL			HPLC-UV	15
Sennoside B	0.07 µg/mL	0.23 µg/mL			HPLC-UV	16
Frangulin A	61.4 ng/mL	122.9 ng/mL	12.3 µg/g	24.6 µg/g	HPLC-UV	10
Frangulin B	77.7 ng/mL	155.3 ng/mL	15.5 µg/g	31.0 µg/g	HPLC-UV	10

Sampling

The literature was not analysed for specific sampling strategies of these food supplements. In general, if these food supplements are sold in retail packages containing 30 to 120 capsules per retail package, the sampling strategy as suggested for red yeast rice food supplements as described in Commission Regulation (EC) No 401/2006 might be applied.

Conclusions

A large number of methods for quantification of aloe-emodin, emodin, aloin A/B and other HADs from the botanical preparations composed of or containing *Aloe, Rheum*, *Cassia* or *Rhamnus* was found in literature. At least part of the methods was (in-house) validated. Methods typically do not include danthrone, which is a synthetic HAD. The relevant HADs are commercially available as analytical standards.

Methods that aim at the determination of the total HAD content without separation of individual compounds are rare. These methods are typically based on NMR techniques.

Extraction of aloe-emodin, emodin, aloin A/B and other HADs from the botanical preparations composed of or containing *Aloe, Rheum*, *Cassia* or *Rhamnus* is often performed by an aqueous solution of methanol or ethanol, optionally acidified with an organic acid. Methods have been developed that use direct injection (after filtration) of the extract, as well as methods that use an additional clean-up step (liquid-liquid extraction or solid phase extraction) to obtain a purified (and concentrated) extract.

Most analytical methods that are available aim at the separation (mostly HPLC) of individual hydroxyanthracene derivatives in combination with UV/DAD or (HR)MS(/MS) detection. For the most important HADs, limits of detection or quantification reported fall in the low ng/mL to µg/mL range in the final extract. This roughly corresponds to 0.1-50 mg/kg in the botanical product. Multi methods that incorporate several HADs (including some glycoside derivatives) are also available. A representative LC-MS/MS method for the analysis of aloe-emodin, emodin, chrysophanol and rhein reports LOQs in the range from 0.63 µg/g for emodin to 1.2 µg/g for aloe-emodin, 2.5 µg/g for rhein and 25 µg/g for chrysophanol. A representative LC-UV method for the analysis of Aloin A and B reports LOQs of 0.23 µg/mL for aloin A and 0.21 µg/mL for aloin B in Aloe extracts.

Based on the similarities between the analytical methods used for the botanical preparations of *Aloe, Rheum*, *Cassia* or *Rhamnus*, it seems plausible that existing methods can be combined or new multi-methods can be developed that cover all relevant HADs for these four groups of botanical preparations. In-house validation and interlaboratory comparison or proficiency testing would be needed to gain insight in the currently achievable performance criteria at the various levels. Furthermore, the inhomogeneity of products as sold on the market needs to be investigated to provide recommendations regarding the amount of sub-sample to be used by the laboratory for extraction.

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Table 4. Analytical methods available for emodin, aloe-emodin and danthron in botanical preparations.

DATE
November 6, 2020

No.	Scope: compounds included	Intended scope plant species	Analytical technique	LOD/LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
1	Emodin, danthron, chrysophanol, physcion	Vegetables: peas, cabbage lettuce, beans; Herbs: grape vine leaves, couch grass root and plantain herb; Liquors	HPLC-PDA GC-MS LC-MS/MS	HPLC (mM/L): Emodin: -/1.38, chrysophanol & physcion: -/2.34 GC-MS LOQs (µmM/L): chrysophanol -/30, physcion -/4	n.i.	Solid materials: freeze-dried, 10-40 g extracted with MeCN in Soxhlet. Liquid materials: acidified with HCL to pH2 and extracted 3 times with EtOAc. All extracts evaporated, re-dissolved in MeCN/H2O, 80/20, injected in HPLC. For LC-MS extraction with acetone/pentane, 50/50, evaporated, re-dissolve in MeCN/H2O, 80/20, injected in LC-MS.	n.i.	(Mueller, Schmitt et al. 1999)
2	Aloe-emodin, emodin, rhein, chrysophanol, physcion, +kaempferol, +6 phenols	Root extracts from *Cassia alata*	HPLC-UV LC-APCI-MS (for identification)	ppm (µg/g) Aloe-emodin: 0.54/- emodin: 0.23/-	ppm (µg/g) Aloe-emodin: 3.06-145 emodin: 2.18-115	10 g extract with 100 mL EtOH for 12h, repeated 2 times, extracts combined, filtered, evaporated, re-dissolved in 10 mL EtOH, diluted 1:1 with H2O. Passed through SPE, eluted with hot EtOH filtered, injected.	Y	(Fernand, Dinh et al. 2008)
3	Aloe-emodin, emodin, rhein, naringin, hesperidin, magnolol, chrysophanol, honikiol	Dachengqi Tang common traditional Chinese medicine formula	HPLC-UV	µg/ml Aloe-emodin: 0.23/0.92 emodin: 0.28/0.83	µg/ml Aloe-emodin: 1.83-146 emodin: 2.2-164	DT granule sample (5 g) ground to fine powder. 100 mg dissolved in 50 ml of 65% (v/ v) aqueous MeOH, then extracted in an ultrasonic water bath (70°C) for 120 min, centrifuged 15 min at 3000 g), supernatant was filtered through a 0.45 µm Millipore membrane filter and injected.	Y	(Tang, Wan et al. 2008)
4	Aloe-emodin, emodin, rhein, chrysophanol, physcion	Rhubarb-based medicines	UPLC-UV	µg/ml Aloe-emodin: 0.04/0.12 emodin: 0.06/0.2	0.4-40 µg/mL	Powdered rhubarb or its preparations (0.15 g) extracted with 25ml MeOH by refluxing for 60min, then filtered. 5 ml filtrate evaporated to dryness. 10 ml of 2M HCl and 20 ml of chloroform were added to dissolve the residue. Solution kept for 1 h on a water bath. The hydrolyzed solution was extracted with 10ml of chloroform 4 times and the combined extract was then evaporated to dryness. The residue transferred into a 50 ml volumetric flask and re-dissolved in MeOH, then filtered through a 0.22 µm filter, injected.	Y	(Wang, Li et al. 2008)
5	Emodin, hypericin, pseudohypericin, hyperforin, hyperoside, rutin, quercetin, quercitrin	Several hypericin-producing species of *Hypericum* Different organs such as leaves, stems and roots of wild-grown plants of *Hypericum hirsutum L.*, *Hypericum maculatum Crantz s. l.*, *Hypericum montanum L.*, *Hypericum tetrapterum Fr.* collected in Slovakia and of *Hypericum perforatum L.* collected in India	LC-MS/MS	Emodin: 0.03/0.1 µg/g	0.01-10 µg/mL	Briefly, the plants were cut into roots, stems and leaves, and were completely air-dried in the oven at 25°C. The dried plant materials were ground to dust under liquid nitrogen. Then, 5 g was extracted independently with 50 ml MeOH/$CHCl_3$ (80/20) by ultra-sonication for 20 min, then filtered. The filtrate was used as the final organic extract. The residue was extracted with 50 ml H2O:MeOH (90:10) by ultra-sonication for 20 min using the same procedure. The sonicated solution was filtered again using Whatman filter paper under vacuum. The filtrate was used as the final aqueous extract. The final extracts were concentrated to 1 ml by rotary evaporation in vacuum at 30 C, and stored in the dark at 20 C till the commencement of the analyses by LC–MS/MS.	Y	(Kusari, Zuhlke et al. 2009)

No.	Scope: compounds included	Intended scope plant species	Analytical technique	LOD/LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
6	Aloe-emodin, emodin, rhein, chrysophanol physcion	Bark of *Rhamnus alpinus L.* (Rhamnaceae)	HPLC-UV	5/10 µM	10-200 µM	3 extraction methods compared: methanol maceration, ultrasonic and supercritical CO2 extraction. MeOH maceration, ultrasonic extraction: sample intake 5 g, extraction with 50 mL of MeOH, then hydrolysis by 6M HCl 30 mL. LLE with EtOAc 20 ml. SFE: 2.5 g of finely triturated bark were placed in a 10 mL disposable SFE extraction cell. The bark sample was held in dynamic extraction at 30°C and 80 MPa for 20 and 60 min at a CO2 flow-rate of 3 mL/min in dynamic mode. The effluent was collected in a sample vial and stored at −20°C until HPLC/UV–vis analysis.	Y (partial)	(Genovese, Tammaro et al. 2010)
7	Emodin, aloe-emodin, rhein, chrysophanol, physcion, madagascin, 3-geranyloxyemodin	*Rhamnus saxatilis Jacq.* and *R. alpinus L., R. pumila Turra*	HPLC-UV	0.3/0.5 µM	0.5-125 µM equivalent to 0.127-50.78 µg/mL	1 g + extraction with n-hexane (first step) and methanol (second step). Extraction procedure not described clear enough.	Y	(Locatelli, Genovese et al. 2012)
8	Aloe-emodin, emodin, rhein, physcion, emodin-8-β-d-glucoside, physcion-8-β-dglucoside, piceid,	*Rhizoma Polygoni Cuspidati*	HPLC-PAD LC-MS (used for confirmation)	HPLC (ng/mL): aloe-emodin: 18/45 (equiv. to 4.5/11 µg/g) emodin: 16.7/20 (equiv. to 4.2/5 µg/g) rhein: 33.3/100 (equiv. to 8/25 µg/g)	HPLC (ng/mL): aloe-emodin: 0.1-10 emodin: 0.09-9 rhein: 0.05-5	0.2 g extracted with 50 ml MeOH, sonicated for 45 min at RT, evaporated at 40°C, re-dissolved in 50 mL PBS, centrifuged, filtered via 0.2 µm, injected in HPLC.	Y (HPLC)	(Xue and Liang 2014)
9	> 300 analytes, including emodin	Cassava	LC-MS/MS	Emodin: 0.2/0.6 µg/kg	28.6-143.2 µg/kg	5 g + 20 mL of MeCN/H2O/HAc, (79/20/1), 90 min extraction, 500 µL of extract diluted with 500 µL of dilution solvent – MeCN/H2O/HAc, (20/79/1), injected.	Y	(Sulyok, Beed et al. 2015) Modified method of Sulyok et al. 2006
10	Emodin, aloe-emodin, rhein, chrysophanol, physcion	Rhubarb (*Rheum palmatum* and *Rheum officinale*)	UPC-SFC-PDA	Emodin & aloe-emodin: 0.42/1.34 µg/mL	Emodin & aloe-emodin: 128-1.6 µg/mL	300 mg of each specimen extracted 5-times with 5 ml MeOH/H2O, 85/15 by sonication for 10 min each. After each repetition the sample was centrifuged (4000 RPM, 10 min), and the supernatants were combined in a 25 ml volumetric flask. The flask was filled to volume, and prior to SFC analysis the solution was filtered through a 0.45 µm membrane filter.	Y	(Aichner and Ganzera 2015)
11	Aloe-emodin, rhein, sennoside A, baicalin, berberine, coptisine, palmatine, resveratrolosi de, wogonin	an-Huang-Xie-Xin-Tang (SHXXT): comprised by three herbal medicines, the *rhizome of Rheum officinale* [or *Rheumtanguticum* (Polygonaceae) (*Dahuangin Chinese*)], the root of *Scutellaria baicalensis (Labiatae*) (*Huangqinin Chinese*), and the rhizome of	HPLC-UV LC-MS (used for confirmation)	µg/mL Aloe-emodin: 0.01/0.03 rhein: 0.01/0.02	µg/mL Aloe-emodin: 0.075-1.5 rhein: 0.1-1.6	5 mg SHXXT-3, dissolved in different MeOH/H2O solutions (5, 50 and 100%) to obtain 0.50 mg/mL solutions. The test solutions were ultrasonicated for 30 min and the individual test solution was filtered over 0.22-µm Millipore filter (Nylon membrane filter), three samples were Prepared (10 µL aliquot) for further HPLC analysis (n = 3).	Y (HPLC)	(Wu, Chang et al. 2016)

No.	Scope: compounds included	Intended scope plant species	Analytical technique	LOD/LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
		Coptischinensis (*Ranunculaceae*) (*Huanglianin Chinese*) in the ratios of 2:1:1 or 1:1:1						
12	Aloe-emodin aloin A/B	*Aloe Vera* raw materials and finished products	HPLC-UV	10/20 ppb (ng/g)	10-500 ppb (ng/g)	Sample intake 0.1 g dry or 1 g of liquid sample. Sample matrix (either liquid or solid form) using stepwise liquid–liquid extraction (water–ethyl acetate–methanol), followed by solvent evaporation and reconstitution.	Y	(Kline, Ritruthai et al. 2017)
13	Emodin, aloe-emodin, chrysophanol, physcion	Rhubarb and its preparations	GC-MS	Emodin: 10/- ng/mL (equiv. to 2 µg/g/-) Aloe-emodin: 20/- ng/mL (equiv. to 4 µg/g/-)	3.2-30 µg/mL	300 mg of dried rhubarb or 500 mg of *Ruyi Jinhuang* + 10 mL EtOAc, filter, 1 mL is sonicated at room T, 15 min, diluted 10 -fold with EtOAc, filter over 0.45 µm filter, inject.	Y	(Dai, Chen et al. 2018)
14	Emodin, allic acid, catechins, epicatechin, polydatin, 2,3,5,4'tetra hydroxystilbene-2-O-β-D-glucoside, resveratrol, physcion	*Radix polygoni multiflori*	UPLC-UV	0.12/0.32 µg/mL (equiv. to 4.8/12.8 µg/g)	Emodin µg/mL: 0.08–100	MMSPD micro-extraction: 25 mg sample + 25 mg adsorbent, 2 min grinding with disorganised silica and 1 mL 150 mM 1-dodecyl-3-methylimidazolium bromide. Then extract solution was centrifuged at 14000 rpm for 10 min and filtered through a 0.45 µm membrane. Two microlitre of the supernatant solution was injected into the UHPLC for analysis.	Y	(Du, Chen et al. 2018)
15	~295 fungal and bacterial secondary metabolites, including emodin	Dried figs	LC-MS/MS	0.1/0.4 µg/kg	2.3-55 µg/kg	Frozen, dried, and cut figs were ground and homogenised, then 5 g + 20 mL of MeCN/H2O/HAc (79/20/1), 90 min extraction, 500 µL of extract diluted with 500 µL of dilution solvent MeCN/H2O/HAc (20/79/1), injected.	Y	(Petric, Sarkanj et al. 2018) Modified method of Malachova et al 2014
16	Aloe-emodin, emodin, rhein, chrysophanol, physcion L-carnitine, nuciferine,	Slimming foods and herbal products	LC-MS/MS	µg/L (equiv. to µg/g) Aloe-emodin: 0.28/1.2 Emodin: 0.19/0.63 Chrysophanol: 7.5/25 Physcion: 10/33 Rhein: 0.83/2.5	µg/L (equiv. to µg/g) Aloe-emodin: 1.25-250 Emodin: 1.25-250 Chrysophanol: 25-2500 Physcion: 40-2000 Rhein: 2.5-250	100 mg extracted with 10 mL MeOH/H2O (50/50), ultrasonification 30 min, centrifugation at 14000 rmp, 5 min, 10-fold dilution with mobile phase, injection.	Y	(Shi, Zhong et al. 2018) Most simple and reliable method!
17	Emodin, physcion, rhein, anthraglycoside B, polydatin, resveratrol	*Polygonum cuspidatum Sieb. et Zucc.* (named Huzhang in china) is a traditional and popular chinese medicinal herb	HPLC-UV	µg/mL Emodin: 0.09/0.24 (equiv. to 9/24 µg/g) Rhein: 0.14/0.44 (equiv. to 14/44 µg/g)	13.32-133.19 µg/mL	The materials of *P. cuspidatum* were dried and powdered to obtain 60-mesh size by grinder. Powders (0.5 g) were extracted by ultrasonication with 50 mL MeOH for 30 min, and the supernatant solutions were filtered through 0.45 µm micropore membrane prior to HPLC analysis.	Y	(Yan, Wang et al. 2018)
18	Emodin, hypericin, pseudohyperi cin, hyperforin, rutin, hyperoside, quercetin	Different organs of 17 *in vitro* cultured *Hypericum* species, along with *H. tomentosum* and *H. tetrapterum* hairy root cultures, and hairy root-derived transgenic plants of	HPLC-DAD LC-MS (LTQ orbitrap)	Emodin: 2/6 ng/ml	20-10000 ng/ml	Species were cut into roots, stems and leaves and powdered to dust in liquid nitrogen. Extraction with 50 ml MeOH/CHCl3 (80/20) by ultrasonication in chilled conditions (≤4 °C) using a Branson B-12 apparatus operating at 20 kHz and 60 W for 20 min. The acquired solution was filtered, and the filtrate was used as the final organic extract. The residue was extracted with 50 ml H2O/MeOH (90/10) by	n.i.	(Nigutova, Kusari et al. 2019)

No.	Scope: compounds included	Intended scope plant species	Analytical technique	LOD/LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
		H. tomentosum				ultrasonication using the same procedure. The sonicated solution was filtered again using Whatman filter paper, lyophilized, and was then extracted with 50 ml methanol, filtered, and the filtrate was used as the final aqueous extract. The final organic and aqueous extracts were concentrated to dryness by rotary evaporation in vacuum at 30°C and stored in the dark at -20°C. Each extract was re-dissolved in 1 ml methanol for LC-MS analysis.		
19	51 analytes: flavonoids, anthraquinones, esters, fatty acids, phenols, catechins. Including: Emodin-8-O-β-D-glucoside	*Tetrastigma hemsleyanum Diels et Gilg (T. hemsleyanums*)	UPLC-Q-Exactive/MS	0.45/2.73 pg on column (=0.15/0.9 µg/mL)	1.8-116 µg/mL	The dried rhizome parts of *T. hemsleyanums* were ground to powder. For each sample, 15 g of rhizome powder was extracted with 75% ethanol reflux for 3 times, 1 h each time. The ethanol extracts were concentrated under reduced pressure evaporated to dryness and then dissolved with 50% acetonitrile of 25.0mL as the stock sample solution. 1.0 mL of the above stock sample solution, adding baicalein with the final concentration of 4.781 µg/mL as the internal standard (IS), was filtered through a 0.22 μm syringe filter to obtain sample solution for qualitative and quantitative analysis.	Y	(Ding, Liu et al. 2019)
20	Emodin, emodin-8-O-β-D-gluco-pyranoside-2,3,5,4′-tetrahydroxy-stilbene- 2-O-β-d-glucoside, ω-hydroxy-emodin, kaempferol	Raw and processed *F. multiflora*	UPLC-Q-TOF-MS	0.02/0.06 µg/mL	Emodin: 0.06-800 µg/mL	The raw and processed *F. multiflora* was crushed into fine powder and passed through a 60 meshes sieve. About 1.0 g powdered F. multiflora samples were immersed into methanol–water solvent in a flask and then ultrasonicated in a water bath at room temperature for an appropriate period. The sample solutions were centrifuged at 13,400 rpm for 10 min. The supernatant was kept at 4°C pending UHPLC-Q-TOF-MS analysis.	Y	(Tao, Zhou et al. 2019)
21	>800 metabolites including emodin	Pig feed samples	LC-MS/MS	0.1/0.4 µg/kg	2.3-55 µg/kg	Sample intake 0.5 g. The samples were placed at darkness to avoid analyte degradation and stored overnight at room temperature to allow the evaporation of the solvent and to establish equilibration between analytes and matrix. After this period, 2 mL of extraction solvent MeCN/H2O/HAc (79:20:1, v/v/v) was added. The samples were extracted for 90 min using rotary shaker and subsequently centrifuged for 2 min at 3000 rpm The extracts were transferred into glass vials using Pasteur pipettes, and 350 µL aliquots were diluted with the same volume of dilution solvent MeCN/H2O/HAc (20:79:1, v/v/v). After appropriate mixing, 5 µL of the diluted extract was injected into the LC–MS/MS system without further pre-treatment. It should be noted that the whole procedure was miniaturized only for validation purposes to decrease the amount of standards needed for spiking. In routine analysis, 5 g of sample is extracted with 20 mL of extraction solvent.	Y	(Khoshal, Novak et al. 2019) Modified mehod of Malachova et al. 2014

No.	Scope: compounds included	Intended scope plant species	Analytical technique	LOD/LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
22	Emodin, physcion, endocrocin, dermolutein, hypericin, skyrin	Complex crude extracts of *Chilean dermocyboid Cortinarii*	HPTLC-DESI-HRMS	n.i.	n.i.	Air dried sample 2 g + 15 mL of acetone in a blender followed by an ultrasonic extraction for 15 min to remove interfering compounds such as fatty acids from the material. After vacuum-supported filtration, the fungal material residue was further extracted twice with 15 mL MeOH each. The resulting extracts were filtrated and dried under reduced pressure using a rotary evaporator. The crude methanolic extracts were re-dissolved in MeOH and directly spotted on the HPTLC plate for chromatographic separation.	n.i.	(Laub, Sendatzki et al. 2020)
23	32 analytes including Aloe-emodin, Emodin, Rhein, Chrysophanol Sennoside A/B	Sanhuang Tablet (SHT), that contains extracts of *Scutellariae Radix* and *Rhei Radix et Rhizoma*, as well as the powder of *Rhei Radix et Rhizoma*	UHPLC-Q-TOF-MS	ng/mL Aloe-emodin: 0.45/1.51 (equiv. to 0.28/0.90 µg/g) Emodin: 0.62/2.08 (equiv. to 0.38/1.24 µg/g) Rhein: 1.24/4.12 (equiv. to 0.76/2.5 µg/g) Chrysophanol 2.53/8.44 (equiv. to 1.5/5.1 µg/g)	ng/mL Aloe-emodin: 43-5505 Emodin: 93.9-3005 Rhein: 19.41-2485 Chrysophanol 42.66-5460	10 ground tablets, sieved and 500 mg extracted 3 times with 10 mL of EtOH/H20 (70/30) in ultrasonic bath for 30 min. Due to differences in content of tablets extracts were diluted 10×, 250× and 400× before analysis.	Y	(Fung, Lang et al. 2017)

Table 5. Analytical methods available for aloin A and aloin B as well as total HAD content in botanical preparations of *Aloe* species.

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical technique	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
1	Aloin	food	Sum of aloin A/B	Column chromatography with UV	n.i.	0.05-50 mg/g	Sample extracted with water from foods containing aloe and the extract is cleaned on disposable cartridge by using methanol-water (55+45).	N (?)	(Yamamoto, Ishikawa et al. 1985) Full text is not accessible
2	Aloin A/B, aloeresin, hydroxyaloin, aloinoside A/B	Beverages	Aloin A/B, aloeresin, hydroxyaloin, aloinoside A/B	HPLC-UV	0.15 ppm (=µg/g)	n.i.	Powdered aloe 1 g mixed with 100 mL of H2O or with H2O-alcohol or absolute EtOH. Centrifuged and diluted 1:4, then filtered via 0.45 µm filter and injected.	N	(Zonta, Bogoni et al. 1995)
3	Aloin A Aloe-emodin	aloe based commercial products (liquids, gels and solids)	Aloin A Aloe-emodin	GC-MS	Aloin A: 0.05/0.1 ppm (=µg/g) Aloe-emodin: 0.005/ 0.01 ppm (=µg/g)	Aloin A: 0.1-20 ppm (=µg/g) Aloe-emodin: 0.01-2 ppm (=µg/g)	1 ml of liquid product or 0.5g of gel or 0.25 g of solid product. Add 0.5 ml alcohol, 1 ml saturated NaCl sol., 2 ml of EtOAc/MeOH, 9/1, vortex for 30 s, centrifuge, organic layer re-extracted with 1 ml of solvent, combine extracts, evaporate, re-dissolve in 0.5 ml of EtOAc/MeOH, 9/1, filter, inject.	Y	(ElSohly, Gul et al. 2004)
4	Aloin A/B	aloe-based products	Aloin A/B	TLC/HPLC-UV	2/- µg/spot	0.5-20 mg/spot	A 1 g portion was accurately weighed and extracted twice with a 5 mL ethanol–water solution (90 + 10, v/v) at room temperature for 5 min with continuous mixing on a shaker. Each extract was filtered through Whatman No. 1 filter paper. Each extract was evaporated under reduced pressure on a rotary evaporator All extracts were stored at −5°C before using. Dried extracts were dissolved in 10 mL of an ethanol–water (90 + 10) mixture. A 1:50 dilution with a solution of 0.1% sodium metabisulfite in water was made before applying to a TLC plate or injecting into the column.	Y	(Ramirez Duron, Ceniceros Almaguer et al. 2008)
5	Aloin A/B, 5-hydroxy-aloin, 7-hydroxy-aloin A/B	Extracts of Aloe plants *A. vera* and *A. ferox*	Aloin A/B, 5-hydroxy-aloin, 7-hydroxy-aloin A/B	Nano-LC-UV-MS	Aloin A/B: 0.4/1.5 µg/ml	1-50 µg/ml	Dried extract, yellow/orange powder, was dissolved in methanol (5 mg/mL), mixed for 10 min in an ultrasonic bath, and centrifuged for 10 min at 2500 rpm. The supernatants of *A. vera* and ferox extracts were diluted at 1/40 and 1/10 ratio, respectively, in 0.02% TFA, 85:15 H2O/ACN v/v and directly injected into the nano-LC system.	Y	(Fanali, Aturki et al. 2010)
6	Aloin A, vitamin C, β-carotene, astaxanthin	*Aloe vera* different foods: peppers, guava, shrimps, carrot	Aloin A	HPLC-UV with microwave-assisted extraction (MAE)	0.54 mg/L	2.0–20 mg/L	5.0 g sample and 100 mL extraction solvent (EtOH) were introduced into the extraction tank, then a magnetic agitation, a condenser and a vacuum pump were equipped. Liquid samples were taken out and the analyte concentration was then measured by HPLC.	Y	(Xiao, Song et al. 2012)
7	Aloin A/B	*Aloe vera* raw materials and finished products	Aloin A/B	HPLC-UV	Aloin A: 0.092/ 0.23 µg/mL Aloin B: 0.087/ 0.21 µg/mL	0.3–50 µg/mL	An extraction procedure using sonication with an acidified solvent (MeOH/HAc 0.1%) was used for solid test materials while liquid test materials only required dilution, if necessary, prior to filtration and analysis.	y	(Brown, Yu et al. 2014)

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical technique	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
	Total HADs	Anthranoid containing herbal drug preparations	Total HADs total hydroxyanthracene glycosides	NA	NA	NA	NA	NA	(Fierens and Corthout 2014) Full text not available
8	Aloin, aloe-emodin, isoalliin, butylphthalide N-methyl-pelletierine, pelletierine, pseudo-pelletierine, chlorogenic acid	Fresh plant samples: red onion, celery, pomegranate, crabapple, potted aloe, mint	Aloin, aloe-emodin	*In vivo* nanospray HRMS	n.i.	n.i.	No sample preparation	N	(Chang, Peng et al. 2015)
9	Aloin A/B, aloe-emodin	*Aloe vera* raw materials and finished products	Aloin A/B, aloe-emodin	HPLC-UV	10/20 ppb (=ng/g)	10-500 ppb (=ng/g)	Sample intake 0.1 g dry or 1 g of liquid sample. Sample matrix (either liquid or solid form) using stepwise liquid–liquid extraction (water–ethyl acetate–methanol), followed by solvent evaporation and reconstitution.	Y	(Kline, Ritruthai et al. 2017)
10	Aloin	Aloe-based formulations	Total aloin	HPLC-UV	0.0053/ 0.0161 µg/ml	5–500 µg/ml	20 tablets + 75 ml MeOH, ultrasonication for 30 min, filtration, dilution up to 100 ml of MeOH. 1 ml diluted up to 50 ml of MeOH and then filtered via 0.45 µm. injected in HPLC.	Y	(Moein, Hajimehdipoor et al. 2017)
11	Aloin	Fresh and dry samples of *Aloe barbadensis* gel and latex	Aloin	HPLC-UV	n.i.	Latex, µg/mL: 162.5–3551.8 Gel, µg/mL: 41.7–2454.8	About 5mg of dry latex and 10 mg of FL were dissolved in PBS (pH 3) to 5 mL. A total of 50 mg of dry gel, 2 g of FG and 100 mg of *A. barbadensis* capsules were all prepared by dissolving in PBS (pH 3) to 10 mL. The commercial aloe juice concentrate was injected directly without dilution. All samples were sonicated for 10 min and filtered through 0.45 µm, then injected.	Y	(Sanchez-Machado, Lopez-Cervantes et al. 2017)
12	Aloin A/B	Aloe plants and aloe-containing beverages, and pharmaceutical preparations	Aloin A/B	Microchip capillary electrophoresis coupled with laser induced fluorescence detection	Aloin A, ng/mL: 7.3/24.5 Aloin B, ng/mL: 7.5/24.9	25–500 ng/mL	For the pharmaceutical preparations, the sugar coatings of capsules were removed, and the residues were finely ground in an electric grinder; for the soft gels, the contents were dried at 80°C before powdered. For the aloe crude drugs, they were all ground into fine powder. For the aloe plants, the fresh leaves were dried at 80◦C overnight and well pulverized. For each sample, a total of 10 mg of finely ground powder was added into 10 mL of methanol/water (20:80, % v/v) solution, and then ultrasonically treated for 10 × 3 min at room temperature. Followed by 12,000 × g centrifugation, the supernatant was then filtered through a 0.22 µm membrane filter before use. For aloe gel-containing drinks, 1 mL aliquot was centrifuged, and supernatant was stored as samples. Each sample analysis was repeated five times.	Y	(Xiao, Bai et al. 2018)

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical technique	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
13	Aloin A/B, 7-hydroxy-aloin, aloinosides	Powdered plant material of *Aloe vera*, *Aloe ferox*	Aloin A/B + total anthranoid content	Band-selective quantitative heteronuclear single quantum correlation spectroscopy (bsqHSQC) (NMR)	Aloin A/B: -/1.63 mg/mL Total antranoids: -/0.815 mg/mL	6.525–52.2 mg/mL	10 g of *Aloe ferox* were suspended in water and heated for 10 min over a water bath. After cooling, the mixture was filtered through a Büchner funnel and extracted five times with 200 mL of EtOAc. Organic phases were combined, rewashed with water and the solvent was evaporated. This procedure was repeated another 4 times before the combined residues were dissolved in a mixture of CHCl3/MeOH (6/1) under heating, and the solution was kept at −20°C over the weekend. The mixture was subsequently filtered through a Büchner funnel, yielding 335 mg of aloin crystals. Purity of the crystals was assessed with NMR using duroquinone as internal standard and resulted to be 93.20%.	Y	(Girreser, Ugolini et al. 2019)

Table 6. Analytical methods available for hydroxyantracene derivatives as well as total HAD content in botanical preparations of *Rheum*, *Cassia*, *Rhamnus*.

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical techn.	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
1	Sennoside A/B	*Cassia angustifolia* leaves and pods	Sennoside A/B	HPLC-UV	Sennoside A: 0.2/- µg/mL Sennoside B: 0.1/- µg/mL	2–50 µg/mL	Leaves (1.0 g) were finely powdered and extracted with hexane (3*25 mL). The hexane extract was discarded and 25 mL of MeOH/H2O (70:30, v/v) was added to the mark, the suspension left overnight at room temperature (25°C) and then further extracted with the (70:30, v/v) mixture (3*25 mL). The extracts were bulked and made up to 100 mL with (70:30, v/v) was. An aliquot (1 mL) of extract was filtered through a sample filtration kit (PTFE; Waters, Milford, USA) and a 10 mL sample was subjected to HPLC analysis. The extraction of pods was performed exactly as described above for leaf samples.	Y	(Bala, Uniyal et al. 2001)
2	Sennoside A/B	*Senna* tablets	Sennoside A/B	HPLC-UV	n.i.	Sennoside A/B: 30–70 µg/ml	Tablets of a senna preparation from a pharmaceutical company were ground to fine powders. 5 mg of the powder were dissolved in 5 ml of sodium bicarbonate solution (1 g in 1000 ml of water). The mixture was sonicated to make sennosides A and B completely dissolve. After filtration through a 0.45 mm Nylon membrane (Whatman).	Y	(Sun and Su 2002)
3	Sennoside A, emodin, rhein, aeoniflorin, naringin, baicalin, baicalein, saikosaponin A	Chinese herbal formula Da-Chai-Hu-Tang	Sennoside A, emodin, rhein, aeoniflorin, naringin, baicalin, baicalein, saikosaponin A	HPLC-UV	n.i.	µg/mL Sennoside A: 5–20 Rhein: 25–100 Emodin: 30–120	0.4 g sample of Da-Chai-Hu-Tang was extracted with 50 mL 70% methanol under ultrasonication for 30 min followed by centrifugation. The extract solution was concentrated to dryness. After adding 1 mL internal standard solution, the herbal preparation extract diluted to 20 mL with 70% methanol.	Y	(Li, Chiu et al. 2006)
4	107 phenolic compounds, including Sennosine A, rhein 8-O-glucoside, rhein 1-O-glucoside, rhein 1-O-(O-acetyl)-glucoside, emodin 1-O-glucoside, emodin 8-O-glucoside, Emodin 8-O-(6'-O-malonyl) glucoside, Aloe-emodin 8-O-(6'-O-acetyl) glucoside, chrysophanol 8-O-β-D-glucoside, chrysophanol 8-O-(6'-O-galloyl)-glucoside	Several rhubarb species: *Rheum officinale, R. palmatum, R. tanguticum, R.franzenbachii, R. hotaoense, R. emodi,*	107 phenolic compounds, including Sennosine A, rhein 8-O-glucoside, rhein 1-O-glucoside, rhein 1-O-(O-acetyl)-glucoside, emodin 1-O-glucoside, emodin 8-O-glucoside, Emodin 8-O-(6'-O-malonyl) glucoside, Aloe-emodin 8-O-(6'-O-acetyl)glucoside, chrysophanol 8-O-β-D-glucoside, chrysophanol 8-O-(6'-O-galloyl)-glucoside	LC-DAD-MS	n.i.	n.i.	For LC/MS analysis, the samples were ground into fine powder (100–150 mesh). An aliquot of 0.25 g was weighed, and extracted with 10 ml of methanol in an ultrasonic water bath at 25 °C for 30 min. The solution was filtered through 0.2-µm membranes before use, and a 5-µl aliquot was injected.	Y	(Ye, Han et al. 2007)

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical techn.	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
5	Sennoside A, Sennoside B,	Crude drugs (*Senna* leaf, *Senna* pods, and rhubarb), crude drug products, Kampo formulations	Sennoside A, Sennoside B	HPLC-UV	Sennoside A/B: 0.01/- µg/ml equiv to 0.5/- µg/g in the sample	Sennoside A/B: 0.02-100 µg/mL	1 g sample dissolved in a solution of methanol–0.2% sodium bicarbonate (7:3, v/v) and applied to the Oasis MAX cartridge. The cartridge was washed with a solution of methanol containing 1% acetic acid. SA and SB were eluted with methanol–water–formic acid (70:30:2, v/v), and the eluate was used as the sample solution for HPLC analysis.	Y	(Yamasaki, Kawaguchi et al. 2010)
6	37 analytes, including Sennoside A/B, Aloe-emodin, Emodin, Rhein, Physcion-8-gucoside, Chrysophanol-8-O-β-D-glucopyranoside	Da-Cheng-Qi decoction, *Cortex Magnoliae officinalis, Fructus Aurantii Immaturus*	37 analytes, including Sennoside A/B, Aloe-emodin, Emodin, Rhein, Physcion-8-gucoside, Chrysophanol-8-O-β-D-glucopyranoside	LC-MS/MS	n.i.	n.i.	15-24 g boiled in 300 mL water until 50% of original volume left. This repeated 2 times. Extracts were combined, 6 g of Na-sulphate was added, mixed, filtered and diluted to 250 mL with H2O, filtered via 0.45 µm, injected.	N Screening method	(Xu, Liu et al. 2010)
7	Sennoside A, Sennoside B,	Food	Sennoside A, Sennoside B	HPLC-UV	µg/mL Sennoside A: 0.8/2.1 Sennoside B: 0.6/2.0	µg/mL Sennoside A: 1.40 - 28 Sennoside B: 1.45 - 29	Extraction using ultrasonifiction. Full extraction procedure not available.	Y	(Xiao, Fang et al. 2011)
8	Sennoside A, emodin-8-O-(60-Omalonyl) glucopyranoside, physcion-8-O-b-D-glucopyranoside, 1-O-galloyl-2-O-cinnamoylglucose, 6-hydroxymusizin-8-O-b-D-glucopyranoside, (1)-catechin, gallic acid 3-O-b-D-glucopyranoside, trans-3,5,40-trihydroxystilbene-40-O-b-D-(200-O-galloyl)-glucopyranoside, 4-(40-hydroxyphenyl)-2butanone-40-O-b-D-(200-O-galloyl-600-O-p-coumaroyl) glucopyranoside	*Radix et Rhizoma Rhei*	Sennoside A, emodin-8-O-(60-Omalonyl) glucopyranoside, physcion-8-O-b-D-glucopyranoside, 1-O-galloyl-2-O-cinnamoylglucose, 6-hydroxymusizin-8-O-b-D-glucopyranoside, (1)-catechin, gallic acid 3-O-b-D-glucopyranoside, trans-3,5,40-trihydroxystilbene-40-O-b-D-(200-O-galloyl)-glucopyranoside, 4-(40-hydroxyphenyl)-2butanone-40-O-b-D-(200-O-galloyl-600-O-p-coumaroyl) glucopyranoside	UPLC-DAD-MS/MS	n.i.	n.i.	Powdered product, 1g, extracted with 100 mL MeOH/H2O (60/40) in ultrasonic cell grinder, ten filtered, evaporated, re-dissolved in MeOH, filtered over 0.22 µm filter and injected.	N Screening method	(Wang, Chen et al. 2011)
9	Palmidin, emodin, rhein glucoside, chrysophanol, gallic acid, desoxyrhapontic in	Raw and processed Rhubarb	Palmidin, emodin, rhein glucoside, chrysophanol, gallic acid, desoxyrhapontic in	HPLC-UV LC-MS (used for identification)	n.i.	n.i.	Ground powdered rhubarb samples passed through a 40-mesh sieve. 0.5 g was extracted ultrasonically with 25 mL MeOH for 30 min. The extract was centrifuged, filtered into a 25 mL flask, and diluted to the mark with MeOH. The solution was	N	(Ni, Song et al. 2012)

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical techn.	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
							filtered through a 0.45 µm filter membrane. A filtrate aliquot (10 mL) was used for HPLC and HPLC-MS analysis.		
10	Frangulins A/B, glucofrangulins A/B	Bark of *Frangula alnus*	Frangulins A/B, glucofrangulins A/B	HPLC-UV & UPLC-UV	HPLC & UPLC: Frangulin A: 61.4/122.9 µg/L (equiv. to 12.3/24.6 µg/g) Frangulin B: 77.7/155.3 µg/L (equiv. to 15.5/31.0 µg/g) glucofrangulins A/B: n.i.	HPLC & UPLC: Frangulin A: 0.46-9.2 µg/mL Frangulin B: 0.475-9.5 µg/mL	100 mg of the milled bark of *F. alnus* was weighed into a 50 mL Falcon tube. 20 mL of extraction solution (320 mL aqueous NaHCO3, 2 g/L, 680 mL MeCN) was added, and extraction performed by ultrasonication for 20 min at 35°C. Next, 30 mL of water, adjusted to pH 2.0, was added. After shaking well, about 3 mL of the extract was filtered through a 0.20 µm syringe filter into a HPLC vial.	Y	(Rosenthal, Wolfram et al. 2014) The only available reliable method
11	Sennoside A, Sennoside B, rhein-8-O-glucoside.	*Sennae fructus, Sennae folium*	Sennoside A, Sennoside B	HPLC-UV	n.i.	mg/mL Sennoside A: 13.4-214 Sennoside B: 12.8-204	0.125 g of the powdered herbal drug in a 50 mL volumetric flask. Add 45 mL of a mixture of 7 vol. of MeOH and 3 vol. of a 0.2 % m/V aq. solution of sodium hydrogen carbonate and extract in an ultrasonic bath for 30 min and fill up to the mark. Filter about 5 mL through a 0.45 µm membrane filter. Apply 2.0 mL of the solution to a 3 mL SPE cartridge (Oasis MAX 3 cc (60 mg) previously conditioned with 2 mL of MeOH and 2 mL of a 0.2 % aq. sol. of sodium hydrogen carbonate. Wash the cartridge with 2 mL of water and 2 mL of MeOH to remove neutral compounds. Wash with 5 mL of a 1 % (V/V) glacial acetic acid solution in MeOH to remove acidic compounds. Discard the washings. Elute with 2 mL of a mixture of MeOH/H2O/FA (70/30/2).	Y	(Rosenthal, Wolfram et al. 2014)
12	64 analytes, including antraquinones: Palmidin, Sennoside A/B, Rhein, Emodin, Chrysophanol, Physcion	Herbal medicine Fufang. *Cortex Magnoliae, Fructus Aurantii Immaturus, Radix et Rhizoma Rhei*	64 analytes, including antraquinones: Palmidin, Sennoside A/B, Rhein, Emodin, Chrysophanol, Physcion	UPLC-HRMS (LTQ-Orbitrap)	n.i.	n.i.	Pulverized samples of *Cortex Magnoliae* Officinalis (12 g) and *Fructus Aurantii Immaturus* (24 g) were extracted with 360 mL of boiling water for 30 min. After these samples were cooled and filtered, Radix et *Rhizoma Rhei* (12 g) was added, and the extract was boiled for 10 min. The aqueous extract was separated by filtration (100 mesh), and 9 g of *Mirabilitum* was dissolved to obtain co-decoction. The co-decoction was concentrated in an evaporating dish and dried under reduced pressure vacuum for 24 h at 50°C to obtain the powder. The dried powder was weighed (0.5 g), mixed with 5mL of 75% ethanol solution, and filtered through a 0.22µm membrane filter.	N	(Cao, Chen et al. 2015)

No.	Scope: compounds included	Intended scope plant species	Individual compounds or sum	Analytical techn.	LOD/ LOQ	Working range	Extraction, clean-up	Valid. Y/N	Ref.
13	Sennoside A/B	Laxative herbs, rhubarbs,*Cassia angustifolia Vahl*., Paidu Yangyan capsule	Sennoside A/B	UPLC-UV	µg/mL Sennoside A: 0.07/0.24 (equiv. to 8.8/30 µg/g) Sennoside B: 0.05/0.20 (equiv. to 6.25/25 µg/g)	µg/mL Sennoside A: 0.74-74 Sennoside B: 0.46-46	200 mg + 25 mL of 0.1% (v/v) NaHCO3 aq. sol., extract for 40 min in ultrasonic bath, filter via 0.22 µm filter and inject.	Y	(Tan, Zhao et al. 2015)
14	32 analytes including Sennoside A/B Aloe-emodin, Emodin, Rhein, Chrysophanol	Sanhuang Tablet (SHT), that contains extracts of *Scutellariae Radix* and *Rhei Radix et Rhizoma*, as well as the powder of *Rhei Radix et Rhizoma*	32 analytes including Sennoside A/B Aloe-emodin, Emodin, Rhein, Chrysophanol	UHPLC-Q-TOF-MS	ng/mL Sennoside A: 1.4/4.7 (equiv. to 0.84/2.8 µg/g) Sennoside B: 1.3/4.3 (equiv. to 0.78/2.6 µg/g)	ng/mL Sennoside A: 40-2560 Sennoside B: 30.47-3900	10 ground tablets, sieved and 500 mg extracted 3 times with 10 mL of EtOH/H20 (70/30) in ultrasonic bath for 30 min. Due to differences in content of tablets extracts were diluted 10×, 250× and 400× before analysis.	Y	(Fung, Lang et al. 2017)
15	Sennoside A/B	Extracts of senna leaves (*Cassia angustifolia*)	Sennoside A/B	HPLC-UV	µg/mL Sennoside A: 1/2 Sennoside B: 2/3	µg/mL Sennoside A: 2-40 Sennoside B: 3-40	Different extraction methods tested: cold percolation, reflux, SFE, UASE, MASE. Basic method in brief, 5 g dried powdered leaves refluxed with 100 mL of MeOH/H2O (80/20) for 5h at 60°C, then filtered, evaporated.	N Not clear	(Dhanani, Singh et al. 2017)
16	Sennoside A, Sennoside B	Senna extracts (*Cassia* genus species)	Sennoside A, Sennoside B	LC-MS	mg/mL Sennoside A: 0.7/2.31 Sennoside B 0.07/0.23	5-40 mg/mL	We adopted and modified the method developed by Ohshima et al. 200 mg of the powdered sample was weighed to the tube. An amount of 5 cm3 of 70% methanol was added and extraction took 20 min in an ultrasonic bath. After centrifugation (10 min at 5000 rpm), the liquid phase was removed, and 2 cm3 of 70% methanol were added to the solid phase and the extraction was repeated for 5 min in an ultrasonic bath. This step was once again repeated. The three obtained extracts were combined and made up to a total volume of 10 cm3 with 70% methanol in a volumetric flask. If needed, the solution was appropriately diluted by mobile phase before HPLC analysis.	N (?) Not clear	(Nesměrák, Kudláček et al. 2020) Modified method of Ohshima Y, Takahashi K (1983) J Chromatogr 258:292