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# Fully automated on-line flow-batch based ultrasound-assisted surfactant-mediated extraction and determination of anthraquinones in medicinal plants

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ABSTRACT

Two easily performed procedures – one sequential injection (SIA) and the other stepwise injection (SWIA) – based on on-line ultrasound-assisted surfactant-mediated extraction followed by spectrophotometric determination of total anthraquinones in medicinal plants are presented. Powdered samples containing *Frangula alnus* (cortex) and *Rubia tinctorum* (roots and rhizomes) were placed in the bottom of an extraction chamber connected to an automatic manifold; the chamber was then immersed into an ultrasonic bath and a solution of Triton X-100 was applied as the extraction medium at 75 °C and under ultrasonication. The extract was submitted on-line for spectrophotometric detection at 435 nm wavelength, and the content of anthraquinones was evaluated using Alizarin as the calibration standard. The linear ranges were up to 0.05 and 0.2 g $L^{-1}$ of Alizarin, and the detection limits, calculated as 3 s of a blank test ($n = 10$), were found to be 0.55 mg $L^{-1}$ for SIA and 4 mg $L^{-1}$ for SWIA, respectively. The relative standard deviation, assessed through ten replicate measurements of real samples, varied in the range 1.1–4.6% for both developed procedures. The suggested procedures were validated according to reference methods. The sampling frequencies were 12 $h^{-1}$ for SIA and 6 $h^{-1}$ for SWIA, respectively.


## 1. Introduction

The use of so-called green analytical methods is an absolutely necessary step to take if the inherent goals of social responsibility in analytical chemistry are to be met [1]. Therefore, an undisputed trend in sample preparation at present is to meet the requirements of green chemistry [2–6]. (a) This can be achieved, for example, by replacing hazardous organic solvents with ionic liquids or surfactants. The role of surfactants in various sample pre-treatment techniques, such as liquid-phase and solid-phase extraction, has been discussed in detail in several review articles [7–11]. (b) Another way to improve analytical methods can be through the automation of the whole procedure [12]. Various flow-based techniques with varying levels of automation have been reported to date. Flow injection analysis (FIA), which exploits the continuous flow of reagents, offers a high sample throughput; however, it consumes relatively large volumes of reagents and produces a great amount of waste. In contrast, sequential injection analysis (SIA), which can be considered as the second generation of FIA, operates with only microliter volumes of both samples and reagents. In general, in SIA the sample and reagent zones are aspirated into a holding coil and forwarded to the detector. The reaction product is formed as a consequence of dispersion of the zones. The flow-batch mode is another solution which prevents the unwanted dispersion of the reaction product [13,14]. Therefore, in this case the analytical signal is higher and very close to a signal obtained in a manually performed mode [15,16]. (c) Ultrasonic irradiation is widely used in a variety of fields, including analytical chemistry [17,18]. Various continuous on-line ultrasound-assisted extraction systems can also be found in the scientific literature [19,20].

Nowadays, people are paying more and more attention to their own health. Medicinal plants which have a laxative effect and can thus improve bowel function have become popular as health care products [21]. They mainly contain anthraquinone compounds, which are widely used for pharmaceutical purposes, such as treatment of kidney and bladder stones [22], and have been shown to possess various therapeutic properties, including antibacterial [23,24], antiviral [25], antifungal [26], antioxidant [24,27], mild sedative [28] and anti-cancer activities [29,30]. Many plant families, such as *Leguminosae*, *Liliaceae*, *Polygonaceae*, *Rubiaceae*, and *Rhamnaceae*, contain anthraquinones [31]. The content of anthraquinone derivatives is often used as a criterion in the quality control of plants used for such medicinal purposes.

In general, methods for anthraquinone determination can be divided into two groups: one is based on the separation of various anthraquinone derivatives using a suitable separation technique and followed by detection of the separated anthraquinones; the second is based on

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**Table 1**
Comparison of the methods for determination of anthraquinones.

| Detection technique | Extraction mode | Extraction medium | On-line | Temperature, °C | US | MW | Vortex | Centrifugation | Sample | Analyte | Linear range | LOD | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CZE-UV | UAE | Ethanol, 60 mL | No | — | 3 h | — | — | — | Chinese herb *Paedicalyx attopevensis* Pierre ex Pitard | (1) 1,3-dihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-β-D-xylosyl(1-6)-β-D-glucoside, (2) 1-hydroxy-2-methoxy-3-hydroxymethyl-9,10-anthraquinone-1-O-β-D-glucoside, (3) 1-methoxy-2-methyl-3-hydroxy-9,10-anthraquinones(rubiadin-1-methylether), (4) l-methoxy-2-formyl-3-hydroxy-9,10-anthraquinone | 4.0–500, 15.8–500, 2.0–500, 4.0–500 (μg $mL^{-1}$) | 0.56, 3.75, 0.31, 0.19 (μg $mL^{-1}$) | [32] |
| pCEC-UV–vis | UAE | Methanol, 30 mL | No | — | + | — | — | 1500 rpm, 5 min | Rhubarb | Rhein, aloe-emodin, emodin, chrysophanol, physcion | 0.2–65, 0.1–30, 0.1–55, 0.5–30, 0.5–55 (μg $mL^{-1}$) | 0.06–0.2 μg $mL^{-1}$ | [34] |
| MEKC–UV | UAE | Methanol, 20.0 mL | No | — | 60 min | — | — | — | *Cassia obtusifolia* (Leguminosae) | Chrysophanol, physcion, emodin, aloe-emodinin, rhein | 5–400, 5–400, 1.5–200, 2.5–300, 2.5–300 (μg $mL^{-1}$) | — | [58] |
| MEC–UV | UAE | 70% methanol | No | — | 30 min | — | — | 1500 rpm, 10 min | *Coptidis rhizoma, Scutellariae radix,* and *Rhei rhizoma* | Coptisine, berberine, palmatine, baicalin, sennoside B, sennoside A, emodin, rhein | 10–30, 50–150, 20–60, 60–180, 20–60, 2.5–20, 2.5–20, 2.5–20 (μg $mL^{-1}$) | 0.17–2.06 μg $mL^{-1}$ | [59] |
| GC–FID, MS | UAE | Methanol, 2.5 mL | No | — | 40 min | — | — | 5000 rpm, 15 min | Radix *Polygoni multiflori* | Aloe-emodin, chrysophanol, emodin, physcion, rhein | 2.0–40.0 μg $mL^{-1}$ | 0.22–0.60 μg $mL^{-1}$ | [35] |
| HPLC–UV, fluorescence | Heating on a gas stove | Water, 5 L | No | Boiling point of water | — | — | — | — | Serum and tissue specimens | Aloe-emodin, rhein, emodin, chrysophanol | 0.04–4.0, 0.01–20.0, 0.01–2.0, 0.01–2.0 (μg $mL^{-1}$) | 0.001–0.04 μg $mL^{-1}$ | [46] |
| HPLC–UV, fluorescence | — | Methanol, 0.5 mL | No | — | — | — | 5 min | 4000 rpm, 5 min | *Aloe vera* extracts and commercial formulations | Aloe emodin | UV: 10.0–1000.0, fluorescence: 2.5–1000.0 (ng $mL^{-1}$) | 3, 0.8 ng $mL^{-1}$ | [40] |
| RP-HPLC–UV, fluorescence | UAE | Methanol/water 90:10, v/v, 25 mL | No | — | 30 min | — | — | 2000 rpm, 5 min | Medicinal plants, pharmaceutical preparations | Aloe-emodin, rhein, emodin, chrysophanol, physcion | 0.09–37.50, 0.07–28.00, 0.08–75.00, 0.14–56.25, 0.14–33.00 (mg $mL^{-1}$) | 3.75, 3.50, 12.50, 11.20, 2.20 (ng $mL^{-1}$) | [37] |
| HPLC–UV | UAE | Methanol | No | — | 45 min | — | — | — | *Frangula rupestris, Frangula alnus* | Aloe-emodin, rhein, emodin, chrysophanol, physcion | 10–200 μM | 3 μM | [24] |
| HPLC–UV | Extraction on an orbital shaker | Ethanol, 100 mL | No | — | — | — | — | — | Root extracts from *Cassia alata* L. | Rhein, kaempferol, aloe-emodin, emodin, chrysophanol, physcion | 13.1–115, 5.27–230, 3.06–145, 2.18–115, 6.04–115, 3.26–105 (μg $g^{-1}$) | 0.23–4.61 μg $g^{-1}$ | [33] |
| HPLC–UV | UAE | 10% Genapol X-080 | — | — | 45 min | | — | 10 min | Rhubarb | Aloe-emodin, rhein, emodin, chrysophanol, physcion | 0.054–2.160, 0.1135–4.5400, 0.085–3.400, 0.1095–4.3800, 0.0315–1.2600 (μg) | — | [53] |
| HPLC–UV | | Methanol | No | — | — | — | — | — | *Cassia fistula* | Rhein, emodine, chrysophanic acid | 2.5–15 mg $mL^{-1}$ | — | [41] |

*(continued on next page)*