Journal of Chromatography A, 1218 (2011) 778–786

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separation of 9,10-anthraquinone derivatives: Evaluation of C18 stationary phases

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ARTICLE INFO

Article history:
Received 11 October 2010
Received in revised form 8 December 2010
Accepted 9 December 2010
Available online 16 December 2010

Keywords:
Stationary phases
Anthraquinoids
Reversed-phase liquid chromatography
Analysis
Historic textile
Dye

ABSTRACT

Chromatographic study of natural products helps to determine their molecular composition and to identify their sources (biological, geographical, etc.). However, identifying anthraquinoids is still a challenge because this chemical family is composed of more than half a thousand molecules. In the present work, a series of C18 stationary phases were systematically studied to evaluate their separation potential for these compounds in high performance reversed phase liquid chromatography (RPLC). The stationary phases with various physicochemical properties were evaluated with respect to the separation of 30 standards. Selectivity of the studied stationary phases and symmetry of the obtained peaks were chosen as the evaluation criteria. Calculations of symmetry and separation capacity parameters have been proposed. These parameters are not dependent on the dimensions of the columns and were used for the classifications of phases. In conclusion, this study is intended to be a guide as to which stationary phases analysts can use for resolving separation of a complex mixture of anthraquinoids.

1. Introduction

The anthraquinone derivatives are important compounds of biological origin. They play some effective role as drugs [1–7], mainly as anti-cancer agents [8–10]. On the other hand some of them are toxic or mutagenic [11,12]. Many anthraquinones are coloured and form coordination complexes with several cations [13–15]. These properties led to their use in food colouration [16–18], textile dyeing and artistic colours preparation [19–21].

Studying anthraquinone derivatives allows people to characterise plant and animal extracts for taxonomy [22] and pharmacology purposes [23,24] as well as to determine composition of dyes from artistic and archaeological objects and to identify their sources [25]. For instance, the knowledge of the chemical composition of materials used for dyeing or painting historical objects conveys deeper knowledge about their history and also helps to choose appropriate treatments for conservation and restoration work. Consequently, scientists need an efficient and accurate analytical approach.

Typical method used to separate and identify components of a mixture is high performance liquid chromatography (HPLC) [6,8,26–33]. Reversed-phase liquid chromatography using organic solvent such as acetonitrile or methanol is one of the most commonly used techniques for separating these compounds. Usually few anthraquinone derivatives are studied by chromatographic methods [29,34] but some works, like that of Hemmateenejad et al. [26], deal with more series. However, that paper is more focused on retention theory than on practical aspects of compounds separation. Hundreds of anthraquinone derivatives are biosynthesised [35,36], so their separation in a single run in defined chromatographic conditions and in acceptable analysis times is very difficult. Consequently, a key problem is how to find a stationary phase allowing an efficient separation of a large number of anthraquinone derivatives.

Anthraquinone derivatives of natural origin have various functional groups in positions R1–R8 (Fig. 1 and Table 1). They cover a wide range of hydrophobicity. Because of the skeleton structure of anthraquinoids and also of the presence of groups with various polarities, reversed phase liquid chromatography (RPLC) is a well adapted technique for their separation. In this type of chromatography, the retention mechanism is mainly based on interactions between the hydrocarbon parts of both the compounds and the stationary phase [37–40].

Identification of anthraquinoids is based on comparison with standard compounds. Besides the most common compounds, their identification remains a problem due to lack of available standards. The contribution of mass spectrometry in the identification of these compounds is of interest. Depending on the type of spectrometer, it is possible to access the exact mass and a characteristic fragmentation. This allows people to get the raw formula, possibly with

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doi:10.1016/j.chroma.2010.12.032

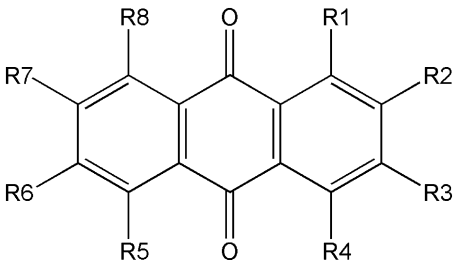

Fig. 1. Anthraquinoid skeleton (see Table 1 for information about R1–R8).

some structural elements. In a previous work [32] it was demonstrated that mass spectrometry allows people to detect co-eluted compounds, but it does not overcome the identification problem for anthraquinonoids with the same mass. Furthermore, in the case of high ratio of signal intensities of overlapping peaks of at least two unknown compounds, the detection of minor compound is difficult. Detection by UV–vis spectroscopy is frequently used for chromatographic quantitative analysis of these compounds and confirming their identity with the reference spectrum. But, in the case of overlapping, it is hard to obtain proper UV–vis spectra of each compound, essential for identification. Moreover, quantification of poorly resolved peaks lacks accuracy. Consequently, a complete separation of the compounds of interest is necessary. Even if some co-eluted target compounds can be separated using mobile phase specific modification, the separation of large sets of compounds using rapid, reproducible and universal tools is needed.

In this study, potentials of various stationary phases were systematically tested in fixed elution conditions in order to determine the more suitable stationary phases for satisfactory separations of the complex mixtures of anthraquinone derivatives.

2. Experimental

2.1. Chemicals and samples

Methanol and acetonitrile were both HPLC grade. They were supplied by Merck (Darmstadt, Germany). Formic acid (HCOOH) was obtained from Carlo Erba (Milan, Italy). Ultrapure water was obtained from Purelab UHQ purification system (Elga, High Wycombe, UK).

Thirty anthraquinone standards (Table 1 and Fig. 1) were used: naturally occurring and synthetic ones in order to have a suitable representation of the diversity of anthraquinoids. Alizarin, emodin, anthraflavic acid and 2-hydroxymethylanthraquinone were purchased from Acros Organics (Geel, Belgium). Physcion, 2,3-dimethylanthraquinone, chrysophanic acid, rhein and aloe-emodin were obtained from Extrasynthese (Genay, France). Purpurin, anthraquinone, tectoquinone, 2-tert-butylanthraquinone, 2-ethylanthraquinone, chrysazin danthron, anthrarufin and 2,3-dimethylquinizarin were from Sigma–Aldrich (Saint Louis, MO, USA). Other standards were obtained from the private collection of Helmut Schweppe (BASF Laboratory, Ludwigshafen, Germany).

Pseudopurpurin and munjistin, for which we had no standards, were extracted from dried madder root (*Rubia tinctorium*).

The standard compounds were dissolved in an acetonitrile/water (50/50) solution at an average concentration of 12 ppm. These solutions were stored at 4 °C. A sample of red coloured wool was taken from a tapestry "The life of the Holy Virgin" dated at the end of 15th Century from Notre-Dame church in Beaune (Burgundy, France). The sample preparation was adapted from protocol elaborated by Sanyova and Reisse [36]. To 2 mg of sample in Eppendorf polypropylene vials with caps 100 μl of mixture of methanol (MeOH)/acetonitrile (MeCN)/4 M hydrofluoric acid (HF) 1:1:2 was added. After sonicating at 45 °C for 30 min, the solu-

Table 1
Functional groups added on anthraquinone skeleton (see Fig. 1) in anthraquinone derivatives.

Compound	Abbreviation	Origin[a]	*M* (g/mol)	R1	R2	R3	R4	R5	R6	R7	R8
Anthraquinone	Anq	N	208	–H	–H	–H	–H	–H	–H	–H	–H
Tectoquinone	Tec	N	222	–H	$-CH_3$	–H	–H	–H	–H	–H	–H
1.3-Dimethyl-anthraquinone	Dma	S	236	$-CH_3$	–H	$-CH_3$	–H	–H	–H	–H	–H
2-Ethyl-anthraquinone	Eta	S	236	–H	$-C_2H_5$	–H	–H	–H	–H	–H	–H
2.3-Dimethyl-anthraquinone	Oma	N	236	–H	$-CH_3$	$-CH_3$	–H	–H	–H	–H	–H
2-Hydroxymethyl-anthraquinone	Hma	S	238	–H	$-CH_2OH$	–H	–H	–H	–H	–H	–H
Anthraflavic acid	Afv	N	240	–H	–OH	–H	–H	–H	–OH	–H	–H
Alizarin	Ali	N	240	–OH	–OH	–H	–H	–H	–H	–H	–H
Anthrarufin	Arf	S	240	–OH	–H	–H	–H	–OH	–H	–H	–H
Danthron (=chrysazin)	Dan	N	240	–OH	–H	–H	–H	–H	–H	–H	–OH
Hystazarin	Hys	S	240	–H	–OH	–OH	–H	–H	–H	–H	–H
Quinizarin	Qza	N	240	–OH	–H	–H	–OH	–H	–H	–H	–H
Xanthopurpurin (=purpuroxanthin)	Xpu	N	240	–OH	–H	–OH	–H	–H	–H	–H	–H
Anthraquinone-2-carboxylic acid	Can	S	252	–H	–COOH	–H	–H	–H	–H	–H	–H
Chrysophanol (=chrysophanic acid)	Chr	N	254	–OH	–H	$-CH_3$	–H	–H	–H	–H	–OH
3-Methoxy-hystazarin	Moh	N	254	–H	–OH	$-OCH_3$	–H	–H	–H	–H	–H
Anthragallol	Agl	N	256	–OH	–OH	–OH	–H	–H	–H	–H	–H
Purpurin	Pur	N	256	–OH	–OH	–H	–OH	–H	–H	–H	–H
2-tert-Butyl-anthraquinone	Bua	S	264	–H	$-C(CH_3)_3$	–H	–H	–H	–H	–H	–H
2.3-Dimethyl-quinizarine	Dmq	S	268	–OH	$-CH_3$	$-CH_3$	–OH	–H	–H	–H	–H
Aloe-emodin	Ale	N	270	–OH	–H	$-CH_2OH$	–H	–H	–H	–H	–OH
Emodin	Emo	N	270	–OH	–H	–OH	–H	–H	$-CH_3$	–H	–OH
Quinalizarin	Qlz	S	272	–OH	–OH	–H	–H	–OH	–H	–H	–OH
Physcion (=parietin)	Phy	N	284	–OH	–H	$-OCH_3$	–H	–H	$-CH_3$	–H	–OH
Rhein	Rhe	N	284	–OH	–H	–COOH	–H	–H	–H	–H	–OH
Flavokermesic acid (=laccaic acid D)	Flk	N	314	$-CH_3$	–COOH	–OH	–H	–H	–OH	–H	–OH
Kermesic acid	Ker	N	330	$-CH_3$	–COOH	–OH	–H	–OH	–OH	–H	–OH
Frangulin	Fra	N	416	–OH	–H	-O-rhamnose	–H	–H	–OH	–H	–OH
Carminic acid	Car	N	492	–OH	-Glucose	–OH	–OH	–H	–OH	–COOH	$-CH_3$
Ruberythric acid	Rba	N	534	–OH	-O-primeverose	–H	–H	–H	–H	–H	–H

[a] N = occurs in nature; S = does not occur in nature.

tion with remaining wool was evaporated to dryness in large volume desiccator over NaOH pellets. The vacuum was obtained using pump equipped with Teflon pistons. The dry residue was solubilised in 100 μl of DMSO on ultrasound bath and, after filtration through PTFE syringe filters, the aliquots were injected into the columns. Because of harmfulness of HF, the appropriated personal safety devices (gloves, goggles) were used. All operations were done under air extractor equipped with acid absorbing filter.

2.2. Instruments and methods

Separations were carried out on a P1000XR quaternary pump system (ThermoFisher, Waltham, USA) equipped with a Spectra system SCM 1000 degasser. The PDA detector was Agilent HP1100 G1315A equipped with the standard cell. The spectra were scanned between 190 and 900 nm. Columns were thermostated at 30 °C by an Igloo-Cil temperature controlled oven (Cluzeau Info Labo, Sainte-Foy-la Grande, France). The software used for data acquisition was AZUR 4.2 with an ULYS port (Datalys, Grenoble, France). Data processing has been done with Microsoft Office Excel software and the statistical software XLStat (Addinsoft, Paris, France). Samples were injected through a Rheodyne injection valve. To save time and to make recognition easier, anthraquinone standards were analysed by injecting them as mixtures of several compounds.

Anthraquinones were analysed on twenty one octadecyl bonded silica stationary phases. Characteristics of selected columns are shown in Table 2. These columns available in our laboratory have previously been used for stationary phase characterisation test of Lesellier and Tchapla [41]. The given physicochemical characteristics (carbon load, specific area, particles size, porosity, bonding type and endcapping) are useful to comprehend properties of these phases. "Bonding type" is related to molecular structure of bonded silica. It corresponds to the monomer or polymer layer graft type as described by Unger [42] and Scott [43]. They are currently called "monomeric" and "polymeric" bonded silica, respectively. Stationary phases from no. 18 to no. 21 listed in Table 2 had a particular bonding type done with polar embedded group alkyl chains. The schematic representations of bonding types are presented in Stella et al. [44]. Chromatographic columns used had various geometric characteristics but these differences were overcome by the introduction of reduced analytical parameters and the relative expression of results. In this way, the comparison is based on the stationary phases in abstraction of particular column size.

Mobile phase used for standards and sample analysis consists of water (A), acetonitrile (B) and formic acid 1% in water (C). The linear gradient was: 0–1 min, 85% A (5% B); 1–66 min, 85–0% A (5–90% B); 66–75 min, 0% A (90% B). The formic acid was maintained at 0.1% in mobile phase introducing 10% of the aqueous solution of HCOOH (C) during analysis. This mobile phase composition was adapted from a previous work [32].

In order to rigorously compare data from columns with different dimensions, we adapted injection volumes and mobile phase flow rates to their geometry. With these conditions, gradient was performed with a flow velocity about 0.16 cm/s, for all columns. That corresponds, for example, to flow rate of 1 ml/min for 4.6 mm internal diameter columns. The injection volume was adjusted to column internal volume, for example: 20 μl for column with 4.6 mm internal diameter.

The historical tapestry sample extract was analysed using *Uptisphere NEC* and *Hypersil Gold* columns. Suitable flow rates and gradient elution were used (Table 3).

Table 2
Characteristics of selected columns (stationary phases).

No.	Stationary phase	Manufacturer	Column length × diameter (mm)	Carbon load (%)	Specific area ($m^2\,g^{-1}$)	Particle size (μm)	Pore diameter (Å)	Bonding type	Endcapping
1	Aquasil C18	Hypersil	250 × 3.0	12	310	5	100	Monolayer mixed phase	Hydrophilic
2	Chromolith RP C18	Merck	200 × 4.6	18	300	Monolith	130	Monolayer	Yes
3	Gammabond RP 18	ES-industries	250 × 4.6		300	5	80	Sandwich structure	No
4	Hypersil BDS C18	Hypersil	100 × 2.1	11	170	3	130	Monolayer	Yes
5	Hypersil Gold	Hypersil	150 × 2.1	10	220	5	175	Monolayer	Yes
6	Nucleodur C18 ISIS	Macherey-Nagel	250 × 4.6	20	340	5	110	Polylayer mixed phase	Yes
7	Nucleosil 100-5 C18 AB	Macherey-Nagel	250 × 4.6	24	350	5	100	Polylayer	Yes
8	Platinium EPS C18	Alltech	250 × 4.6	5	200	5	100	Monolayer	No
9	Purospher star RPC18e	Merck	250 × 4.6	17	330	5	120	Polylayer	Yes
10	Ultracarb	Phenomenex	150 × 4.6	22	370	5	90	Monolayer	Yes
11	Uptisphere HDO	Interchim	150 × 2.0	17	310	5	120	Monolayer	Special
12	Uptisphere HSC	Interchim	150 × 2.0	20	310	5	120	Monolayer	Yes
13	Uptisphere NEC	Interchim	150 × 2.0	16	320	5	120	Monolayer	No
14	Uptisphere ODB	Interchim	150 × 2.0	18	344	5	120	Monolayer	Yes
15	Uptisphere TF	Interchim	150 × 2.0	17	~320	5	120	Polylayer	Yes
16	Zorbax 300SB C18	Agilent	250 × 4.6	10	180	5	300	Monolayer	No
17	Zorbax RX C18	Agilent	250 × 4.6	12	180	5	80	Monolayer	No
18	Nucleosil C18 Nautilus	Macherey-Nagel	150 × 4.6	16	350	5	100	Monolayer polar-embedded	Yes
19	Supelco LC-ABZ	Supelco	250 × 4.6	12	170	5	100	Polylayer amide-embedded	Yes
20	Stability BS-C23	CIL	250 × 4.6		350	5	100	Quaternary ammonium-embedded	Special
21	Uptisphere PLP	Interchim	150 × 2.0	14	320	5	120	Monolayer embedded groups	Yes

Table 3
Chromatographic conditions for the sample analysis.

Stationary phase	Uptisphere NEC	Hypersil Gold
Length × internal diameter (mm)	150 × 2.0	150 × 4.6
Flow (ml/min)	0.2	1.0
Equilibration (min)	16	17
Injection volume (μl)	5	20
% B	min	min
5	0	0
5	2.3	2.7
60	83.7	88.5
60	85.7	90.7

3. Results and discussions

3.1. Selection of C18 stationary phases

Stationary phase selection influences analytical method development. The most commonly used stationary phases in RP-LC are alkyl-bonded silica phases, in particular, octadecyl bonded phases. Referring to literature [44–47], there is a wide set of available C18 stationary phases displaying very different behaviours. But available technical information for these phases is not sufficient to predict which phase is the best for a particular separation (as presented in Table 2). To evaluate the chromatographic properties of stationary phases, tests and characterisation methods have been performed [41,44–53]. Among these methods, the test of Lesellier and Tchapla [41] gives a glimpse of both polar site accessibility and steric hindrance selectivity [52,54] of stationary phases. Using bivariate distribution of Lesellier and Tchapla, we have chosen stationary phases, available in our laboratory, for this study. They were expected to give different separations for anthraquinoids' molecules which have various polar and non-polar groups and present different conformations.

The 30 anthraquinone derivative standards were injected on selected stationary phases and resulting chromatograms were analysed. These analyses revealed that no polar embedded stationary phase allows elution of all anthraquinone standards. Moreover, we did not observe peaks for molecules with a carboxylic acid group like carminic acid, kermesic acid, flavokermesic acid, and anthraquinone-2-carboxylic acid. This may be due to a strong or an irreversible interaction between this group and polar embedded groups of stationary phases in selected conditions. Under low pH conditions, embedded groups containing nitrogen may display some ionic interaction with the acidic analytes as described before by Buszewski et al. [55]. Moreover, the presence of probable residual, post-reactional, amino groups could be contributing to the retention of these acidic compounds, as Czajkowska and Jaroniec noticed [56]. In addition, in the case of *Uptisphere PLP* column, no peak was observed with compounds having an -O-glycoside group like ruberythric acid, and frangulin. Thus, polar embedded stationary phases are not convenient for studying anthraquinoids of either a carboxylic acid group or an -O-glycoside group. These types of anthraquinoids are frequent in natural extracts. Consequently, the polar-embedded stationary phases were not considered for further evaluation.

Two chromatographic parameters were retained as evaluation criteria: peak symmetry and stationary phase separation capacity, because they are related to both the detectability of peaks and the chromatographic resolution. In fact, asymmetry of peaks affects chromatographic efficiency [57,58]. Moreover, minor compounds with wide, asymmetric peaks are usually weakly detected or not detected at all.

3.2. Symmetry parameter

Since distortions of chromatographic peaks can affect the separation it is important to determine the extent of distortion. This can be achieved by calculation of the peak asymmetry factor or tailing factor. The tailing factor of each peak (*TF*) has been calculated following US pharmacopeia according to Eq. (1):

$$TF = \frac{WL_5 + WR_5}{2 \times WL_5} \quad (1)$$

A line perpendicular to the base line is dropped from the peak apex and the widths of the two peak halves are evaluated at 5% at the peak height. WL_5 is the left width (distance between the center line of the peak and the back side of the chromatographic curve) and WR_5 is the right width (distance between the center line of the peak and the front side of the chromatographic curve) of the peak.

Peak asymmetry describes the shape of chromatographic peaks and gives information on the efficiencies of stationary phases for some anthraquinoid compounds. When the *TF* value is far from 1, there is a peak distortion and stationary phase efficiency drops. Important peak tailing (*i.e.* $TF \geq 1.5$) occurs when chemical surface groups or surface impurities of stationary phase display interactions other than pure hydrophobic interaction with the studied compounds. For each stationary phase, tailing factors were calculated (Eq. (1)) and plotted as scattergrams (Fig. 2). Each scattergram shows distribution of tailing factor values on *Y*-axis and average tailing factor, represented by a horizontal line, for a given phase. Compounds with the same tailing value are side by side on the same *Y*-axis. For *Hypersil Gold* (no. 5), the tailing factor values are the less scattered and the average tailing factor is equal to 1. On the other hand, we note that stationary phase *Zorbax 300 SB C18* (no. 16) shows the highest average tailing factor. Considering both physical and chemical properties of these stationary phases, this difference in tailing mainly comes from the purity of silica base, the bonding density and the endcapping process.

Fig. 2 shows also that there always exists at least one compound with a tailing value greater than 1.5 for every column. The extreme point for each scattergram does not represent the same compound but it is frequently purpurin, alizarin, quinalizarin, or anthragallol. We note that all these anthraquinoid compounds, frequently present in dyes, have hydroxyl groups in both R1 and R2 positions. This configuration gives high capacity to form complexes, then generates undesired interactions with chemical surface groups or surface impurities of stationary phase and causes tailing of peaks. The quinizarin, used as tailing marker by some chromatographers [59], form a symmetrical peak on almost all stationary phases.

These scattergrams (Fig. 2) allow us to discriminate stationary phases in terms of the average tailing of the set of standards on one hand, and of distribution of tailing factor values on the other hand. But classification taking in account only the average tailing factor values is not sufficient to indicate whether for instance *Nucleodur C18 ISIS* (no. 6) gives a better general peak symmetry than *Chromolith RP C18* (no. 2). Thus, we need a tool to classify these phases using single value, characterizing the peak symmetry on a given stationary phase. Considering average tailing factor $\overline{TF}$, standard deviation of this value σ_{TF}, and a reference value 1 corresponding to the perfect tailing factor value, we assumed that a symmetry parameter $d(TF;1)$ could be calculated. Inspired by statistical calculations of distances like chi-square test [60], we developed the following Eq. (2):

$$d(TF;1) = \sqrt{\sigma_{TF}^2 + (\overline{TF} - 1)^2} \quad (2)$$

The parameter $d(TF;1)$ takes into account the gap existing between the tailing factor of each compound from the studied set and the perfect symmetry (value 1). The lower this value, the more

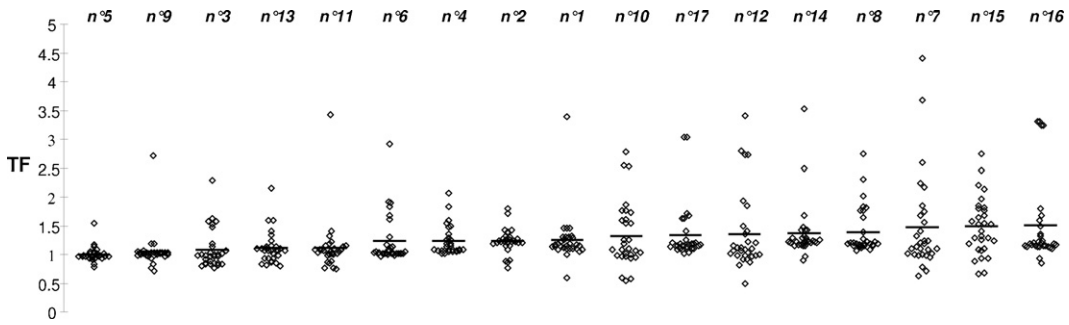

Fig. 2. Scattergrams of tailing factor (TF) values for evaluated stationary phases (numbered). Horizontal lines represent the average values of tailing factor.

satisfying the stationary phase is. Consequently, a classification of stationary phases according to their symmetry factor was made. Results for each stationary phase are given in Table 4. It appears that *Hypersil Gold* (column no. 5) has the lowest value, what has also been seen from former ranking (Fig. 2). So, it is an excellent stationary phase from the symmetry parameter point of view. The silica of *Hypersil* phases has a great purity, so metallic or other impurities do not interact with functional groups of molecules. *Uptisphere NEC* (column no. 13), *Chromolith RP C18* (column no. 2), *Purospher Star RP C18*e (column no. 9), *Gammabond RP 18* (column no. 3) and *Hypersil BDS* (column no. 4), are also interesting stationary phases for analyses of anthraquinones or sets of compounds with various functional groups. Their symmetry parameters are below 0.4, so we give them the rank "A" in this classification.

3.3. Separation capacity parameter

Separation capacity parameter is a measure of the degree of separation of two neighbouring peaks. Retention of each anthraquinone derivative (X) has been normalised to the retention of the anthraquinone standard (Anq) in order to compare columns with different geometries efficiently. Relative retention coefficients ($Rt_{X/Anq}$) were calculated according to Eq. (3),

$$Rt_{X/Anq} = \frac{t_{G(X)} - t_0}{t_{G(Anq)} - t_0} \quad (3)$$

where $t_{G(X)}$ and $t_{G(Anq)}$ are the retention time of a compound and that of anthraquinone in a gradient elution system respectively. We have chosen anthraquinone as a reference because it has only H in positions R1–R8 (Fig. 1). The dead time (t_0) of each column was determined by injecting pure acetonitrile.

Table 4
Calculated symmetry parameter data for stationary phases.

Stationary phase	No.	Symmetry parameter d(As;1)	Rank
Hypersil Gold	5	0.13	A+
Uptisphere NEC	13	0.30	A
Chromolith RP C18	2	0.31	A
Purospher star RP C18e	9	0.33	A
Hypersil BDS C18	4	0.34	A
Gammabond RP 18	3	0.35	A
Nucleodur C18 ISIS	6	0.47	
Uptisphere HDO	11	0.48	
Aquasil C18	1	0.50	
Platinium EPS C18	8	0.57	
Zorbax RX C18	17	0.60	
Uptisphere ODB	14	0.61	
Ultracarb	10	0.65	
Uptisphere TF	15	0.70	
Uptisphere HSC	12	0.78	
Zorbax 300SB C18	16	0.89	
Nucleosil 100-5 C18 AB	7	0.96	

Relative retention coefficients on stationary phases excluding polar-embedded are presented in Table 5. The retention order for anthraquinone-derivatives on each stationary phase is obtained by classifying them according to increasing retention coefficient. As expected, it appears that elution order is not exactly the same for all these phases, which are all octadecyl phases. For instance, results obtained with *Uptisphere HSC* and *Uptisphere TF* (stationary phases no. 12 and no. 15 respectively) show a different elution order for the following couples Moh–Can, Xpu–Rhe, Eta–Chr, Phy–Bua. In fact, *Uptisphere HSC* (no. 12) exhibits a monolayer alkyl bonding surface while *Uptisphere TF* (no. 15) exhibits a polylayer bonding surface. We have seen that anthraquinoid elution orders vary slightly for studied C18 stationary phases. Now, we have to consider separation of peaks for these phases. In this work, we looked for the stationary phase that gives the best selectivity for the 30 standards. Ratio of time differences between two neighbouring apexes over the peak width ($\delta t/\omega$) is considered as a separation capacity parameter for each stationary phase. This parameter is proportional to chromatographic resolution (Rs) that is not affected by column length in our gradient elution conditions and is considered as suitable by chromatographers if its value is at least equal to 1.5 [58,61]. For each stationary phase, separation capacity parameters for pairs of closely eluting compounds were calculated. Poorly separated compounds for a given stationary phase were indicated by a "x" mark in Table 6. As it can be seen in Table 6, no phase can reach a perfect separation for all compounds. According to Table 6, *Gammabond RP 18* (no. 3) Exhibits 17 critical pairs of peaks, which is the highest number. So, this phase is the least suitable for the proposed application. However, the *Uptisphere HSC*, *Chromolith RP C18*, *Zorbax RX C18*, and *Uptisphere NEC* phases (phases no. 12, no. 2, no. 17, no. 13 respectively) generated the smallest number of critical pairs. The latter set of stationary phases is classified as rank "A". Moreover, the phase with the best separation capacity is *Uptisphere NEC* (no. 13); it yields only eight critical pairs: Afv–Ker, Ker–Flk, Moh–Ali, Moh–Can, Can–Ali, Xpu–Qlz, Fra–Rhe, and Qza–Arf.

The frequency of occurrence of critical pairs is given on the right of Table 6. We note that couples Ker–Flk and Moh–Ali are the most persistent. In the first couple, present in natural dyes of insect type (*Kermes* spp., *Porphyrophora* spp. *Dactylopius coccus*), the molecules differ only by a hydroxyl group in position R5. This means that the absence of the hydroxyl group at position R5 of kermesic acid does not affect its retention on C18 stationary phases. Only *Nucleodur ISIS* phase (no. 6) is able to really distinguish kermesic acid (Ker) from flavokermesic acid (Flk). In fact, this phase is a mixed octadecyl/hydroxylated one. It displays additional interactions with hydroxyl groups of kermesic acid (Ker) and retains it more than it does for flavokermesic acid (Flk). In the case of alizarin (Ali) and 3-methoxyhystazarine (Moh), only *Hypersil BDS C18*, *Platinium EPS C18*, and *Uptisphere HSC* (respectively columns no. 4, no. 8, and no. 12) yield a satisfactory separation. Consequently, separation of specific pairs of anthraquinoid compounds can be carried out with a stationary phase chosen from Table 6.

Table 5
Retention coefficients ($Rt_{X/Anq}$) of studied anthraquinoids with stationary phases numbered 1 to no. 17.

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	No. 16	No.17
Car	0.40	0.30	0.34	0.31	0.30	0.32	0.23	0.40	0.31	0.32	0.38	0.35	0.39	0.36	0.31	0.32	0.28
Rba	0.50	0.45	0.45	0.46	0.52	0.43	0.40	0.53	0.44	0.45	0.51	0.47	0.51	0.49	0.52	0.46	0.43
Afv	0.67	0.57	0.63	0.55	0.60	0.59	0.52	0.67	0.60	0.59	0.65	0.60	0.63	0.59	0.61	0.58	0.56
Ker	0.66	0.58	0.65	0.59	0.63	0.84	0.53	0.68	0.59	0.61	0.66	0.60	0.63	0.61	0.68	0.62	0.56
Flk	0.66	0.58	0.65	0.60	0.64	0.79	0.53	0.68	0.60	0.61	0.66	0.61	0.64	0.62	0.69	0.62	0.56
Hys	0.69	0.62	0.65	0.62	0.66	0.64	0.58	0.70	0.65	0.66	0.70	0.65	0.67	0.64	0.67	0.63	0.62
Agl	0.71	0.65	0.66	0.64	0.69	0.68	0.52	0.74	0.67	0.59	0.72	0.67	0.69	0.66	0.71	0.66	0.65
Hma	0.77	0.68	0.69	0.65	0.70	0.70	0.64	0.78	0.70	0.69	0.72	0.70	0.73	0.70	0.72	0.68	0.68
Ali	0.83	0.75	0.77	0.73	0.76	0.79	0.73	0.84	0.79	0.77	0.82	0.76	0.79	0.77	0.81	0.75	0.76
Can	0.82	0.76	0.76	0.76	0.78	0.83	0.73	0.85	0.78	0.77	0.82	0.77	0.80	0.77	0.85	0.77	0.75
Moh	0.83	0.75	0.77	0.74	0.77	0.78	0.72	0.85	0.78	0.77	0.82	0.78	0.80	0.77	0.81	0.76	0.76
Ale	0.85	0.77	0.77	0.75	0.78	0.80	0.74	0.87	0.80	0.78	0.84	0.79	0.82	0.77	0.84	0.77	0.77
Fra	0.86	0.81	0.81	0.85	0.86	0.80	0.78	0.94	0.80	0.81	0.87	0.80	0.85	0.81	0.92	0.84	0.79
Qlz	0.92	0.82	0.82	0.81	0.83	0.91	0.80	0.94	0.88	0.82	0.91	0.82	0.85	0.82	0.94	0.83	0.81
Rhe	0.87	0.86	0.88	0.83	0.86	0.94	0.87	0.90	0.82	0.85	0.87	0.86	0.88	0.86	0.94	0.85	0.86
Xpu	0.90	0.85	0.86	0.84	0.87	0.89	0.83	0.92	0.87	0.86	0.91	0.87	0.88	0.85	0.92	0.85	0.85
Pur	0.92	0.95	1.00	0.85	0.87	0.92	0.94	0.96	0.89	0.96	0.92	0.98	0.90	0.87	0.95	0.86	0.87
Emo	1.03	0.95	1.01	0.92	0.92	1.04	0.95	1.07	1.01	0.96	1.05	0.99	0.98	0.95	1.12	0.92	0.97
Anq	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dan	1.07	1.04	1.08	1.02	1.00	1.09	1.05	1.08	1.08	1.04	1.07	1.07	1.06	1.03	1.12	1.00	1.07
Tec	1.10	1.07	1.12	1.06	1.03	1.12	1.08	1.12	1.11	1.07	1.12	1.11	1.09	1.06	1.16	1.04	1.10
Qza	1.12	1.11	1.16	1.09	1.06	1.16	1.17	1.14	1.14	1.15	1.14	1.14	1.12	1.09	1.21	1.06	1.14
Oma	1.18	1.12	1.19	1.11	1.08	1.20	1.16	1.20	1.20	1.15	1.20	1.16	1.13	1.11	1.28	1.08	1.15
Arf	1.13	1.16	1.21	1.15	1.11	1.17	1.19	1.14	1.15	1.16	1.15	1.20	1.16	1.15	1.24	1.13	1.19
Chr	1.18	1.18	1.22	1.22	1.13	1.23	1.22	1.21	1.21	1.17	1.21	1.21	1.17	1.16	1.32	1.14	1.21
Eta	1.18	1.18	1.24	1.23	1.14	1.22	1.19	1.21	1.21	1.18	1.22	1.23	1.19	1.16	1.28	1.16	1.20
Dma	1.23	1.23	1.32	1.29	1.18	1.28	1.26	1.25	1.27	1.23	1.27	1.28	1.23	1.22	1.33	1.20	1.27
Phy	1.27	1.28	1.29	1.33	1.21	1.33	1.37	1.31	1.29	1.27	1.30	1.31	1.28	1.22	1.52	1.23	1.31
Bua	1.30	1.34	1.41	1.41	1.29	1.35	1.33	1.32	1.35	1.32	1.38	1.39	1.33	1.33	1.42	1.32	1.35
Dmq	1.41	1.47	1.54	1.54	1.36	1.54	1.68	1.46	1.47	1.49	1.46	1.50	1.45	1.32	1.82	1.37	1.52

3.4. Complementarity of stationary phases

Columns no. 5, no. 13, no. 2, no. 9, no. 4 and no. 3 show the best characteristics from the peak symmetry point of view, and columns no. 13, no. 17, no. 12 and no. 2 exhibit the best separation capacity. For analyses of samples with compounds such as quinalizarin that often display undesirable interactions with stationary phases, use of *Hypersil Gold* (no. 5) is required. For efficient separation of numerous anthraquinoids, *Uptisphere NEC* (no. 13) is the more suitable. In case of specific critical pairs, another column can be used to complement the *Uptisphere NEC* phase. We suggest alternative stationary phases for *Uptisphere NEC* (no. 13) in Table 7, that is *Nucleodur C18 ISIS* (no. 6) stationary phase because it improves chromatographic separation resolving six of the eight critical pairs of *Uptisphere NEC* (no. 13). *Hypersil BDS* (no. 4) would also be used to separate peaks of Qza–Arf and Moh–Ali, unresolved using *Uptisphere NEC* (no. 13) and *Nucleodur C18 ISIS* (no. 6) *phases*. This phase resolves five of the eight critical pairs of *Uptisphere NEC* (no. 13) and shows good peak symmetry.

For instance, an extract from a real sample was analysed using the best stationary phase of each previous evaluated parameter that is *Uptisphere NEC* and *Hypersil Gold* stationary phases. The chromatographic conditions for each of them are given in Table 3. The chromatograms obtained with UV–vis detection at 254 nm are presented in Fig. 3. Nine compounds were identified from the extract (Table 8) and there was an impurity of the solvent called "A" on the chromatograms. Nordamnacanthal and Munjistin were characterized in a previous work [32] by liquid chromatography tandem mass spectrometry. Pseudopurpurin and lucidine primeveroside were characterized in this work comparing their UV–vis spectra to reference spectra in literature [62,63] and flavokermesic acid glycoside was characterized in this work by mass spectrometry. Chromatogram from *Uptisphere NEC* column shows an efficient separation for almost all constituents of the sample. This phase properly separates the critical pair of flavokermesic acid glycoside (Flk-gly) and carminic acid (Car). However, the couple pseudopurpurin–munjistin (Psp–Mun) is co-eluted. This weak separation is partially due to the tailing of pseudopurpurin peak. Chromatogram recorded using *Hypersil Gold* column presents exceptional peak symmetry, particularly for peak of pseudopurpurin. Thus, it allows a satisfactory separation of the couple pseudopurpurin–munjistin (Psp–Mun). Unfortunately, this phase does not separate flavokermesic acid glycoside (Flk-gly) from carminic acid (Car). *Uptisphere NEC* is more retentive than *Hypersil Gold*. This result is interesting for an analysis of anthraquinoids with a mass spectrometric detection. In this case, a mobile phase with a large fraction of organic modifier can be used in order to improve electrospray ionization and sensitivity of detection [32].

In conclusion, we advise that a real sample like this extract should be analysed with complementary columns *Uptisphere NEC* and *Hypersil Gold*.

4. Conclusion

We attempted to find optimal C18 stationary phases able to analyse a large set of standard anthraquinoid compounds in a single run. This study allowed us to compare the behaviour of octadecyl bonded phases with respect to analyses of anthraquinone derivatives. No polar embedded stationary phases were retained because they cannot ensure the elution of all anthraquinoid derivatives. Two chromatographic parameters were used as criteria of choice: symmetry of peaks and separation capacity of stationary phase. First, using a suggested symmetry parameter $d(TF;1)$ including average tailing factor and its standard deviation, we showed that *Hypersil* phases (no. 4 and no. 5) give the best symmetry in anthraquinoid's analysis. For selectivity aspect, *Uptisphere NEC* phase (no. 13) develops a minimum of critical pairs of anthraquinone derivatives. This phase gives the most satisfying chromatographic separation of anthraquinoid compounds. However, none of the studied stationary phases is able to separate all standards in linear gradient conditions and in acceptable analysis time. Further adjustments of separation could be performed using mobile phase composi-

Table 6
Separation capacity: number of critical pairs on each stationary phase and frequency of occurrence of each critical pairs.

Pairs of peaks	Stationary phases no.																	Occurrence
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Afv–Flk	x							x	x		x	x						5
Flk–Hys								x								x		2
Dan–Ker																	x	1
Dan–Flk																	x	1
Afv–Ker	x	x	x				x	x	x		x	x	x					9
Ker–Flk	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	16
Ker–Hys			x	x				x							x	x		5
Ker–Can						x												1
Flk–Ali						x												1
Flk–Moh						x												1
Flk–Ale						x												1
Afv–Agl							x			x								2
Afv–Hys			x															1
Agl–Flk			x				x											2
Agl–Hma				x	x						x				x			4
Agl–Afv							x			x								2
Moh–Ale		x	x	x		x					x	x		x				7
Moh–Ali	x	x	x		x	x	x		x	x	x		x	x	x	x	x	14
Moh–Can		x	x	x	x		x	x	x	x	x	x	x	x				12
Rhe–Pur															x			1
Can–Ali	x	x	x				x	x		x	x	x	x	x			x	11
Can–Ale		x	x	x	x		x							x	x	x		8
Ali–Ale			x			x			x					x				4
Ali–Fra						x			x									2
Ale–Fra	x					x			x			x						4
Pur–Fra				x	x													2
Xpu–Rhe				x														1
Xpu–Qlz		x	x		x				x	x	x	x	x	x		x	x	11
Xpu–Pur				x	x						x					x		4
Xpu–Fra				x	x										x			3
Qlz–Pur	x				x	x			x		x			x	x	x	x	9
Rhe–Qlz															x			1
Pur–Anq		x	x				x			x		x						5
Anq–Emo			x									x						2
Fra–Rhe			x	x						x	x		x		x	x	x	8
Qlz–Fra								x								x		2
Emo–Pur			x															1
Emo–Dan					x			x							x	x		4
Qza–Arf	x					x	x	x	x	x			x			x		8
Oma–Chr	x		x		x			x	x		x					x		7
Oma–Eta	x					x	x	x						x	x			6
Eta–Chr	x	x		x	x	x		x	x	x				x			x	10
Phy–Dma														x				1
Chr–Dma															x			1
Qza–Oma				x						x								2
Phy–Bua								x										1
Dmq–Bua														x				1
Total pairs	12	10	17	13	14	13	12	14	13	12	13	10	8	13	13	14	9	
Separation rank		A										A	A+				A	

Table 7
Complementary separation capacity: phases resolving critical pairs of *Uptisphere NEC* phase (no. 13).

	Stationary phase no.															
	6	4	15	5	1	8	12	14	16	17	2	7	9	3	10	11
Critical pairs of phase no. 13																
Fra–Rhe	R			R	R	R	R	R			R	R	R			
Qza–Arf		R	R	R			R	R		R	R			R		R
Afv–Ker	R	R	R	R				R	R	R					R	
Xpu–Qlz	R	R	R		R	R						R				
Can–Ali	R	R	R	R					R				R			
Moh–Can	R		R		R				R	R						
Moh–Ali		R				R	R									
Ker–Flk	R															
Number of pairs resolved (R)	6	5	5	4	3	3	3	3	3	3	2	2	2	1	1	1

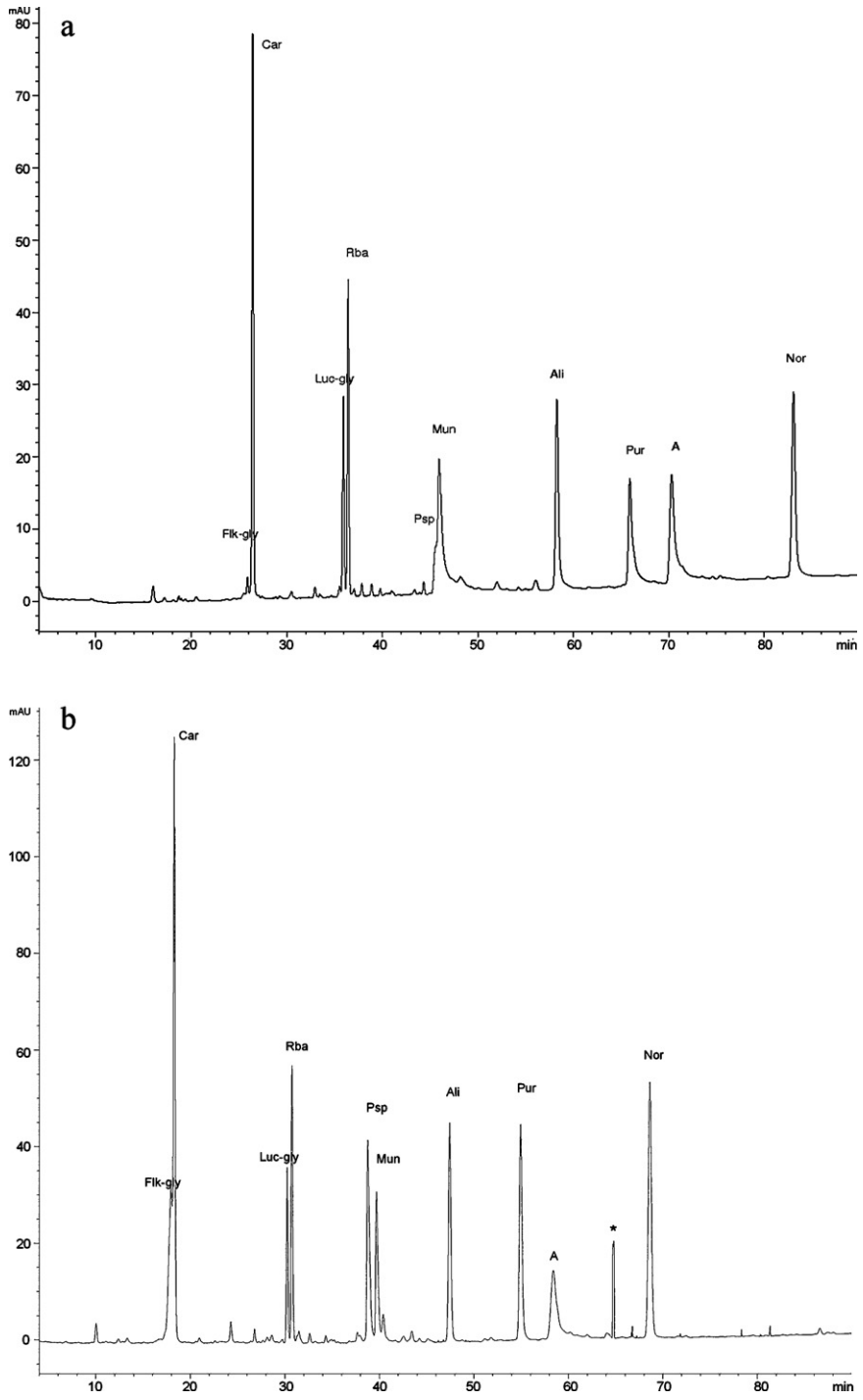

Fig. 3. Chromatograms of historic sample obtained with UV detection at 254 nm with (a) *Uptisphere NEC* phase and (b) *Hypersil Gold* phase. Peak "A" is a formic acid impurity accumulated. * indicates a spike in (b).

Table 8
Identified anthraquinone derivatives from historical textile sample.

Compound	Abbreviation	*M* (g/mol)	R1	R2	R3	R4	R5	R6	R7	R8
Alizarin	Ali	240	–OH	–OH	–H	–H	–H	–H	–H	–H
Purpurin	Pur	256	–OH	–OH	–H	–OH	–H	–H	–H	–H
Nordamnacanthal	Nor	268	–OH	–CHO	–OH	–H	–H	–H	–H	–H
Munjistin	Mun	284	–OH	–COOH	–OH	–H	–H	–H	–H	–H
Pseudopurpurin	Psp	300	–OH	–COOH	–OH	–OH	–H	–H	–H	–H
Flavokermesic acid glycoside	Flk-gly	476	$-CH_3$	–COOH	–OH	–H	–H	–OH	-Glucose	–OH
Carminic acid	Car	492	–OH	-Glucose	–OH	–OH	–H	–OH	–COOH	$-CH_3$
Ruberythric acid	Rba	534	–OH	-O-prime-verose	–H	–H	–H	–H	–H	–H
Lucidin primeveroside	Luc-gly	564	–OH	$-CH_2OH$	-O-prime-verose	–H	–H	–H	–H	–H

tion (organic modifier) and gradient modifications (multi-slope, isocratic).

In conclusion, we advise analysts to use *Hypersil Gold* (no. 5) and *Uptisphere NEC* (no. 13) phases for their analyses of mixture containing anthraquinoids. In some particular cases where all peaks are not resolved, use of complementary phases is suggested in Table 7.

References

[1] S. Enayat, S. Banerjee, Food Chem. 116 (2009) 23.
[2] A. Golcu, D. Gitmisoglu, M. Dolaz, S. Serin, Asian J. Chem. 21 (2009) 321.
[3] G.C. Yen, P.D. Duh, D.Y. Chuang, Food Chem. 70 (2000) 437.
[4] J. Koyama, I. Morita, K. Tagahara, M. Ogata, T. Mukainaka, H. Tokuda, H. Nishino, Cancer Lett. 170 (2001) 15.
[5] D.L. Barnard, D.W. Fairbairn, K.L. Oneill, T.L. Gage, R.W. Sidwell, Antiviral Res. 28 (1995) 317.
[6] V.E. Fernand, D.T. Dinh, S.J. Washington, S.O. Fakayode, J.N. Losso, R.O. van Ravenswaay, I.M. Warner, Talanta 74 (2008) 896.
[7] Y. Li, T. Wu, J. Zhu, L. Wan, Q. Yu, X. Li, Z. Cheng, C. Guo, J. Pharm. Biomed. Anal. 52 (2010) 597.
[8] S. Eksborg, H. Ehrsson, J. Pharm. Biomed. Anal. 2 (1984) 297.
[9] M. Ye, J. Han, H.B. Chen, J.H. Zheng, D. Guo, J. Am. Soc. Mass Spectrom. 18 (2007) 82.
[10] X. Liang, L. Zhang, X. Zhang, W. Dai, H. Li, L. Hu, H. Liu, J. Su, W. Zhang, J. Pharm. Biomed. Anal. 51 (2010) 565.
[11] G.C. Barbee, M.M. Santer, W.R. McClain, Crop Prot. 29 (2010) 506.
[12] Y. Wang, J.W. Chen, L.K. Ge, D.G. Wang, X.Y. Cai, L.P. Huang, C. Hao, Dyes Pigm. 83 (2009) 276.
[13] S. Karaderi, D. Bilgic, Rev. Anal. Chem. 27 (2008) 251.
[14] I. Surowiec, W. Nowik, M. Trojanowicz, Microchim. Acta 162 (2008) 393.
[15] D. Bilgic, S. Karaderi, I. Bapli, Rev. Anal. Chem. 26 (2007) 99.
[16] F. Miyamoto, M. Saeki, M. Kamijo, H. Kanda, T. Nakaoka, M. Nishijima, Y. Ito, R. Takeshita, Eisei Kagaku-Jpn. J. Toxicol. Environ. Health 37 (1991) 542.
[17] E. Andrzejewska, Rocz Panstw Zakl Hig 32 (1981) 315.
[18] S. Dixit, S.K. Khanna, M. Das, J. AOAC Int. 91 (2008) 1387.
[19] X. Zhang, I. Good, R. Laursen, J. Archaeol. Sci. 35 (2008) 1095.
[20] I. Surowiec, B. Szostek, M. Trojanowicz, J. Sep. Sci. 30 (2007) 2070.
[21] I. Petroviciu, F. Albu, A. Medvedovici, Microchem. J. 95 (2010) 247.
[22] J.W. Fairbairn, F.J. El-Muhtadi, Phytochemistry 11 (1972) 263.
[23] Y. Igarashi, M.E. Trujillo, E. Martínez-Molina, S. Yanase, S. Miyanaga, T. Obata, H. Sakurai, I. Saiki, T. Fujita, T. Furumai, Bioorg. Med. Chem. Lett. 17 (2007) 3702.
[24] P. Xiao, L. He, L. Wang, J. Ethnopharmacol. 10 (1984) 275.
[25] J.H. Hofenk De Graaff, The Colorful Past, London, Archetype Books, 2004.
[26] B. Hemmateenejad, M. Shamsipur, A. Safavi, H. Sharghi, A.A. Amiri, Talanta 77 (2008) 351.
[27] S. Genovese, F. Tammaro, L. Menghini, G. Carlucci, F. Epifano, M. Locatelli, Phytochem. Anal. 21 (2010) 261.
[28] I. Surowiec, J. Orska-Gawryś, M. Biesaga, M. Trojanowicz, M. Hutta, R. Halko, K. Urbaniak-Walczak, Anal. Lett. 36 (2003) 1211.
[29] Z. Zhang, W. Sun, P. Luo, L. Wu, L. Ye, H. Zhang, J. Pharm. Biomed. Anal. 48 (2008) 980.
[30] I. Karapanagiotis, Y. Chryssoulakis, Ann. Chim. 95 (2005) 1.
[31] G.C.H. Derksen, H.A.G. Niederländer, T.A. van Beek, J. Chromatogr. A 978 (2002) 119.
[32] L. Rafaëlly, S. Héron, W. Nowik, A. Tchapla, Dyes Pigm. 77 (2008) 191.
[33] L. Zhu, H. Li, Y. Liang, X. Wang, H. Xie, T. Zhang, Y. Ito, Sep. Purif. Technol. 70 (2009) 147.
[34] S.O. Mueller, M. Schmitt, W. Dekant, H. Stopper, J. Schlatter, P. Schreier, W.K. Lutz, Food Chem. Toxicol. 37 (1999) 481.
[35] R.H. Thomson, Naturally Occurring Quinones. III: Recent Advances, Chapman & Hall, London, 1987.
[36] J. Sanyova, J. Reisse, J. Cult. Herit. 7 (2006) 229.
[37] J.L. Rafferty, J.I. Siepmann, M.R. Schure, J. Chromatogr. A 1216 (2009) 2320.
[38] M. Turowski, T. Morimoto, K. Kimata, H. Monde, T. Ikegami, K. Hosoya, N. Tanaka, J. Chromatogr. A 911 (2001) 177.
[39] A. Rizzi, in: E. Katz, R. Eksteen, P. Schoenmakers, N. Miller (Eds.), Handbook of HPLC, Marcel Dekker, New York, 1998, p. 28.
[40] C. Horváth, W.R. Melander, E. Heftmann, Chapter 3: Theory of Chromatography, Elsevier, 1983, p. A27.
[41] E. Lesellier, A. Tchapla, J. Chromatogr. A 1100 (2005) 45.
[42] K.K. Unger, J. Chromatogr. Libr. 16 (1979) 102.
[43] R.P.W. Scott, Sep. Sci. Series, John Wiley, Chichester, vol. 4 (1993) 146.
[44] C. Stella, S. Rudaz, J.L. Veuthey, A. Tchapla, Chromatographia 53 (2001) S113.
[45] B. Buszewski, S. Kowalska, T. Kowalkowski, K. Rozpedowska, M. Michel, T. Jonsson, J. Chromatogr. B 845 (2007) 253.
[46] N.S. Wilson, J.W. Dolan, L.R. Snyder, P.W. Carr, L.C. Sander, J. Chromatogr. A 961 (2002) 217.
[47] N.S. Wilson, J. Gilroy, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 1026 (2004) 91.
[48] K. Kimata, J. Chromatogr. Sci. 27 (1989) 721.
[49] E. Lesellier, C. West, A. Tchapla, J. Chromatogr. A 1111 (2006) 62.
[50] C. West, L. Fougère, E. Lesellier, J. Chromatogr. A 1189 (2008) 227.
[51] J. Layne, J. Chromatogr. A 957 (2002) 149.
[52] L.C. Sander, S.A. Wise, LCGC Int. 3 (1990) 24.
[53] J.J. Gilroy, J.W. Dolan, P.W. Carr, L.R. Snyder, J. Chromatogr. A 1026 (2004) 77.
[54] L.C. Sander, S.A. Wise, J. Chromatogr. A 656 (1993) 335.
[55] B. Buszewski, J. Schmid, K. Albert, E. Bayer, J. Chromatogr. 552 (1991) 415.
[56] T. Czajkowska, M. Jaroniec, J. Chromatogr. A 762 (1997) 147.
[57] C. Stella, S. Rudaz, J.-Y. Gauvrit, P. Lantéri, A. Huteau, A. Tchapla, J.-L. Veuthey, J. Pharm. Biomed. Anal. 43 (2007) 89.
[58] P.J. Schoenmakers, J.K. Strasters, Á. Bartha, J. Chromatogr. 458 (1988) 355.
[59] L.C. Sander, S.A. Wise, J. Sep. Sci. 26 (2003) 283.
[60] C. Liteanu, I. Rica, Statistical Theory and Methodology of Trace Analysis, John Wiley and Sons, Chichester, R. A. C. a. M. R. Masson, 1980.
[61] R. Rosset, M. Caude, A. Jardy, Manuel pratique de la chromatographie en phase liquide, Paris, Masson, 1982.
[62] I. Boldizsar, Z. Szűcs, Zs. Füzfai, I. Molnár-Perl, J. Chromatogr. A 1133 (2006) 259.
[63] G. Cuoco, C. Mathe, P. Archier, F. Chemat, C. Vieillescazes, Ultrason. Sonochem. 16 (2009) 75.