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Determination of polyphenols in pear pulp matrix by solvent extraction and liquid chromatography with UV-Vis detection

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Abstract

A new analytical method for the determination of polyphenolic compounds in pear pulp was here developed. The procedure consisted of solvent extraction for the recovery of analytes and further quantification by reversed-phase high-performance liquid chromatography with multidetection by UV-Vis molecular absorption spectroscopy. Preliminary studies were focused on establishing a straightforward extraction procedure of soluble compounds using organic and hydro-organic media. Dimethylsulfoxide was selected as the most efficient extraction solvent for the diverse polyphenol families. The chromatographic separation relied on a methanol gradient which was optimized by experimental design. Figures of merit were established under the selected experimental conditions using synthetic standards and pear extracts. In general, repeatabilities of peak areas were better than 3%, detection limits were in the order of magnitude of 0.1 mg L^{-1} and quantitative recoveries were about 100%. The method was applied to analyze commercial pears of various origins being chlorogenic, neochlorogenic and gallic acids, arbutin and catechin some of the most abundant compounds. Differences in the polyphenol composition among pear varieties were found to be relevant. As a result, such compounds may result in potential descriptors of varietal characteristics.

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Introduction

Polyphenols are secondary metabolites of plants, often classified into four main families according to the number of phenol rings that they contain as well as the structural elements that bind these rings together as follows[1-3]: (i) Phenolic acids, comprising two subclasses of hydroxybenzoic and hydroxycinnamic acids. They account for 30% of total dietary polyphenols and, in general, cinnamic derivatives are more abundant in fruits than benzoic ones.[4] (ii) Flavonoids, consisting of two aromatic rings linked by three carbon atoms that form an oxygenated heterocycle. Flavonoids account for 60% of total dietary polyphenols and can be divided into six subclasses, namely: flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols.[5-7] (iii) Stilbenes, characterized by a double-bond connecting the phenolic rings. Despite stilbenes are found in low quantities in the human diet, their nutritional significance is very important.[4,8] (iv) Lignans, a minor class of polyphenols consisting of two phenylpropane units. The main food source of lignans is linseed although they are also found at lower concentrations in cereals, fruits and vegetables.[4,8] All these types of compounds occur in plants as single molecules, the so-called aglycones, or conjugated with one or more sugar residues thus resulting in the corresponding glycosides.[1]

Polyphenols contribute significantly to organoleptic and nutritional properties of fruits. Sensory features such as color, bitterness and astringency strongly depend on the content of such substances.[2,9,10] Regarding biological effects, polyphenols have widely been studied because of some beneficial properties on the human health, such as antioxidant, antiviral, anti-inflammatory, anti-allergic, antibiotic, anti-carcinogenic and cardioprotective activities.[2,11-15] Besides, some polyphenolic compounds have been recognized as potential chemotaxonomic markers since they are typical or specific of some fruit species.[16-20] For instance, phloretin and phlorizin are characteristic of apples, punicalagins (ellagic acid derivatives) of pomegranate, naringenin derivatives of citric fruits, and arbutin of pears.[21-24]

Pear is a species belonging to the genus *Pyrus* of the family *Rosaceae*. There are many pear varieties cultivated around the world that differ in size, shape, texture, color, flavor, etc.[25] Due to the diversity of polyphenolic compounds in pear, some concerns on recovery, separation, identification and quantification remain unresolved. Although, first studies of polyphenols in pears began in 1980, a complete characterization is still under development and qualitative and quantitative data is limited. The identification of some major phenolic components of pears, such as arbutin, chlorogenic, caffeic, p-coumaric, and p-coumaroyl quinic acids, (+)-catechin, (-)-epicatechin, and flavonol glycosides, has been

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reported in several publications.[26-30] From these studies, it has been found out that amounts (and diversity) of phenolic compounds in pear skin are much higher than in pulp analogously as it occurs with other fruits.[31,32] Besides, other factors such as variety, ripeness, harvest date, storage conditions, etc. can cause noticeable quantitative differences in the compositional profiles.[33,34]

The extraction of soluble polyphenols have often been carried out with organic solvents such as methanol and ethanol or some organic/water mixtures.[35] In some cases, the extraction process has been combined with acid treatment to hydrolyze glycoside bonds, thus yielding the corresponding aglycones.[35,36] Also, acid cleavage has been applied to analyze hydrolyzable tannins. For dealing with non-extractable derivatives such as proanthocyanidins, oxidative treatment to break interflavan bonds can be utilized.[37,38] New strategies based on extractive-solid phase extraction have also been introduced to recover polyphenols from various vegetable matrices.[39]

Liquid chromatography (HPLC and UHPLC) is the most used analytical technique for quantification of phenolic compounds in pears and related products.[26-30] Analogously to other food samples, the separation is commonly carried out in a C18 column using suitable elution gradients.[29,32,35,40-42] Mobile phases consist of diluted aqueous solutions of organic acids (e.g., formic or acetic acids) and organic solvents such as methanol or acetonitrile. For detection, UV-Vis spectroscopy at 280 nm is used as a representative wavelength of all polyphenolic compounds. If multidetection is available, e.g., using a diode-array spectrophotometer (DAS), other characteristic wavelengths can also be considered for a more specific detection of some families of compounds, such as 370 nm for flavonoids or 520 nm for anthocyanins.[43] The fluorescence of polyphenols can also be exploited for a more selective and sensitive detection of some analytes.[43] Alternatively, polyphenols display redox (oxidizable) properties that open up great analytical possibilities via electrochemical monitoring.[44] To gain both selectivity and detectability mass spectrometry (MS) can be coupled. Besides, MS is an excellent choice for unambiguous identification of phenolic compounds. To date, however, the number of publications related to LC-MS is limited.[21,26,44-47] Other less extended analytical methods are based on gas chromatography (GC) and capillary electrophoresis (CE).[48-50]

In this paper, pears of some varieties with high commercial impact such as Conference, Blanquilla, Ercolini and Alejandrina have been analyzed and compared. The study has first been addressed to the development of a new analytical method to determine polyphenolic compounds in pear pulp. The method combines a sample treatment by solvent extraction, HPLC separation using a C18

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column and DAS detection. In order to evaluate the overall extraction yield and optimize the chromatographic conditions multiobjective responses and experimental design approaches have been proposed. Figures of merit have been established under the selected conditions. The most relevant polyphenols found in pear pulp have been identified. Compositional data of different pear varieties have also been evaluated to try to find potential markers characteristic of each class.

This new method aims at providing simplicity, speed, reduced cost and acceptable analytical parameters to the issue of the determination of polyphenol in pear matrices. The introduction of chemometric methods for experimental design and data analysis has contributed to achieve a more efficient optimization of the HPLC-UV method. In this way, figures of merit have been improved significantly with respect to other published HPLC-UV methods. For instance, the analysis time have been reduced 2- to 5-fold approximately, and the separation quality has been enhanced in terms of peak resolution, recovery and accuracy. It is obvious that some powerful analytical methods have recently been proposed for similar purposes such as those based on (U)HPLC-MS using, for instance, QTOF and orbitrap analyzers[36,45]. The performance of such instruments is excellent although the cost may be unacceptable for some small laboratories, especially when dealing with routine analysis. In a similar way, sophisticated applications based on comprehensive two-dimensional liquid chromatography and nano-HPLC have been described for polyphenol profiling[32,40]. However, these approaches seem to be less user-friendly and the generation and interpretation of results may be complex.

Materials and methods

Chemicals and standards

Unless specified, analytical grade reagents were used. Milli-Q water (Millipore, Milford, MA, USA), formic acid (99% w/w, from Merck, Darmstadt, Germany) and HPLC grade methanol (MeOH, from Panreac, Barcelona, Spain) were the components for the preparation of the mobile phase. 4-O-cafeolquinic, caftaric, caffeic, chlorogenic, coumaric, 2,5-dihydroxybenzoic, ellagic, ferulic, gallic, 4-hydroxibenzoic, homovanillic, neochlorogenic, protocatechuic and sinapinic acids, protocatechuic and syringic aldehydes, apigenin, arbutin, (+)-catechin, cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin chloride, (-)-epicatechin, epigallocatechin, fisetin, ethyl gallate, isorhamnetin, kaempferol, myricetin, morin, piceid, procyanidin B1, quercetin-3-galactoside, quercetin-3-glucoside, quercitrin, *t*-resveratrol, rutin, taxifolin and tyrosol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Individual stock

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solutions of each polyphenol were prepared at a concentration of 5 mg mL^{-1} in dimethylsulfoxide (DMSO, from Merck, Darmstadt, Germany). Stock solutions were stored in dark vials at 4°C. Polyphenol standard mixtures for the assessment of quality parameters and quantification, with concentrations ranging from 0.2 to 200 μg mL^{-1}, were prepared in DMSO by diluting stock solutions. Solutions were stable under refrigeration, at least, for a month.

Apparatus and instruments

The chromatographic system consisted of an Agilent 1100 Series HPLC instrument equipped with a G1311A quaternary pump, a G1379A degasser, a G1392A autosampler, a G1315B diode-array detector furnished with a 13-μL flow cell and an Agilent Chemstation for data acquisition and analysis (Rev. A 10.02), all of them from Agilent Technologies (Waldbronn, Germany). The analytical column used was a 100 × 4.6 mm i.d., 2.6 μm, Kinetex C18 reversed-phase, with a 4.0 × 3.0 mm i.d. guard column of the same material (Phenomenex, Torrance, CA, USA).

Auxiliary apparatus to be used in the sample treatment were as follows: a sonication bath Branson 5510 (Branson Ultrasonics, Danbury, CO, USA), Rotanta RS 460 centrifuge (Hettich, Germany) and Cyberscan 2500 pH meter (Eutech Instruments, Singapore, Singapore) with a Hamilton pH electrode (Bonaduz, Switzerland).

Sample preparation

Pears of different varieties (*conference*, *blanquilla*, *ercolini*, *alejandrina and Williams*) were purchased in retail stores. Pears were peeled, cut in small dices and mashed. Immediately, 0.2 g of sample were weighed (precision ± 0.0001 g) in vial and soluble polyphenols were extracted with 1 mL of DMSO. The sample mixtures were sonicated for 10 min and then centrifuged at 3050 × g for 10 min. 0.5 mL of clean supernatant solutions were taken for chromatographic analysis. Prior to injection, extracts were filtered through 0.45 μm PTFE membranes (Scharlab, Barcelona, Spain). Extracts stored at 4°C were stable for one month.

Chromatographic determination

Chromatographic separations were carried out by elution gradient using an aqueous phase (solvent A) consisting of 0.1% (v/v) formic acid and methanol (solvent B). The elution gradient was as follows: time

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range 0 to 3 min, 5%→ 25% B (linear increase); 3 to 6 min, 25% B (constant); 6 to 9 min, 25 → 37% B (linear increase); 9 to 13 min, 37% B (constant); 13 to 18 min, 37 → 54% B (linear increase); 18 to 22 min, 54% B (constant); 22 to 26 min, 54 → 95% B (linear increase); 26 to 29 min, 95% B (constant); 29 to 29.1 min, 95 → 5% B (linear decrease); 29.1 to 35 min, 5% B (constant, equilibration step). The flow rate was 1 mL min^{-1} and the injected volume 10 µL. Chromatograms were recorded at 280, 310, 370 and 520 nm. Benzoic acids, phenyl alcohols, stilbenes, tannins and flavonols were detected at 280 nm, cinnamic acids at 310 nm, and the rest of flavonoids at 370 nm (see details on Table 2).

Results and discussion

Optimization of the extraction

Soluble polyphenols were extracted from mashed pear samples using organic solvents or hydroorganic mixture solutions. The optimal working conditions were defined as those leading to the highest overall recovery from a series of model analytes belonging to the different families. Some important experimental variables to be assayed as they affected the extraction yield were pH, extraction time, and type of solvent.

The effect of pH was studied in the range 1 to 13 using several aqueous solutions including 0.1 M HCl, 0.1 M formic acid, 0.01 M $H_2PO_4^-$ / HPO_4^{2-} (pH = 7), 0.01 M tetraborate (pH = 9.2) and 0.1 M NaOH. Due to the wide variety of physicochemical features of the diverse families of polyphenols, some fractions were efficiently extracted at acid pH while others were better recovered in basic media. Results obtained showed that extraction at neutral pH was preferred as an overall compromise to maximize the recovery of components of different classes.

The performance of the extraction in aqueous solutions was compared with various pure solvents (MeOH, ethanol and DMSO) and aqueous-organic mixtures (MeOH/water 50:50, MeOH/water 90:10, DMSO/water 50:50 and DMSO/water 90:10, all of them expressed in percentage of volume, *V:V*). In this study, samples (0.2 g) were treated with 1 mL of the mentioned solvents. Results for various phenolic acids, flavanols and flavones were simultaneously considered to calculate the average extraction percentage. As shown in Fig 1, the best overall recoveries were obtained with pure DMSO. It should be mentioned that, in general, the extraction percentages of phenolic acids and some flavonoids in water and in DMSO were similar. However, less polar components (particularly some flavonoids such as quercetin

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179 and kaempherol) were better recovered in DMSO so that this solvent finally was chosen for further
180 experiments.
181 The influence of the sonication time on the extraction was evaluated in the range 10 to 40 min.
182 In all the cases, results were similar so that it was concluded that 10 min were sufficient to extract the
183 analytes.
184 The estimation of the percentage of each polyphenol that was extracted from the pear matrices
185 was carefully evaluated. The principal difficulty arose in the fact that variable amounts of polyphenols
186 occurred naturally in the samples, hence suitable blanks reproducing the complexity of the pear matrix
187 were not available. In these circumstances, the chromatograms of the pear extracts provided profiles
188 displaying the background phenolic contents. The determination of the extraction percentage was carried
189 out taking these profiles as the basal reference. Here, a series of 6 mashed samples were subjected to the
190 extraction procedure and analyzed by HPLC thus representing the phenolic background. These sample
191 extracts were conveniently spiked with standards at 10 mg L^{-1} of each analyte and were further injected
192 into the HPLC (post-added samples). The increase in the signals with respect to the background
193 corresponded to a recovery of 100%. Another series of 6 mashed samples were spiked prior extraction
194 with 10 mg L^{-1} of each analyte and were extracted analogously (pre-added samples). For each compound,
195 the ratio in the net peak areas of pre- and post-added samples expressed the extraction recovery. Results
196 given in Table 1 indicated that recoveries for most of the analytes under study were about 60 to 85%.
197 Extractions were more favorable for simpler molecules such as benzoic and cinnamic acid while
198 decreased for less polar flavonoids. The variability of these results, expressed as RSD%, ranged between
199 1 to 9%. From this assay, it was concluded that the extraction of analytes belonging to the different
200 families was satisfactory although the recovery percentage has to be accounted in the quantification of
201 analytes in pears.
202

203 **Optimization of the separation conditions**

204 A chromatographic method previously developed for the determination of polyphenols in wines was here
205 adapted to the analysis of pear samples.[43,51] With respect to these former methods, although many
206 polyphenols occur in both wine and pear matrices, some of them are new and characteristics of pears. On
207 the contrary, various polyphenolic chemical descriptors of wine are irrelevant in pear. The separation was
208 re-optimized focusing on the pear matrix in order to fulfil an acceptable separation of remarkable

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209 compounds, especially those more prominent in pears such as arbutin, chrologenic acid and its isomers.
210 Experimental design was used to facilitate the optimization under the compromise of high resolution and
211 reduced analysis time. In this way, excellent separations can be gained from a reduced set of experiments.

212 The systematic optimization of chromatographic separations is almost indispensable when
213 dealing with complex samples as those encountered in the field of food analysis. This step is crucial when
214 using UV-Vis detection but it should not be underestimated in MS counterparts. Our idea was to run an
215 experimental plan as a way of avoiding large and costly series of trial-and-error assays. The starting point
216 was the definition of the optimization criteria, here implemented as mathematical expressions to take into
217 account all desired objectives simultaneously. In our case, a single objective response may be insufficient
218 to express the optimal situation of the chromatographic separation so that multicriteria approaches are
219 recommended. Here, objectives such as peak resolution, number of compounds separated and analysis
220 time were combined in an objective response function given as a mathematical expression. This was
221 achieved according to product functions, in particular, Derringer desirability functions written as the
222 following generic expression: $D = \Pi(d_i)^{1/n}$ where D is the overall response, d_i represents each individual
223 desirability and n is the number of responses considered.

224 A standard mixture consisting of 18 polyphenols belonging to several families, each at 5 μg mL^{-}
225 ^{1}, was used for the optimization of the separation gradient. As described in the experimental section, 0.1%
226 formic acid (solvent A) and methanol (solvent B) were used to create the elution profiles. The elution
227 gradient was established according to an experimental design consisting of 2 factors, namely, initial
228 gradient time (t_0) and MeOH percentage (MeOH%) as similar strategies provided highly successful
229 results in other chromatographic optimizations.[52-54] Preliminary studies involving linear gradients with
230 various slopes and initial MeOH% evidenced the complexity of the separation, with several coelutions of
231 analytes with similar chemical structures and physicochemical properties. As a result, more complex
232 gradient profiles were required to resolve the mixture of standards. Here, three isocratic steps were
233 included within the gradient profile with low ~22, intermediate ~33 and high ~50 MeOH percentages.
234 Each isocratic range intended the improvement of the separation of overlapping compounds of
235 hydroxybenzoic, hydroxycinnamic and flavone families, respectively. The experimental design was
236 conducted at 2 and 3 levels for t_0 and %MeOH, respectively. 6 experiments were run to complete the
237 design (see Fig. 2a). The best chromatographic separation was defined as that reaching a full resolution of

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analytes in the minimum analysis time. Fig. 2b shows the separations of the standard mixture depending on the gradient profiles. Best results corresponded to run 3 so it was preliminarily chosen.

In order to confirm the performance of the selected elution gradient in the presence of the sample matrix, the same optimization strategy was applied to pear extracts. Chromatograms showed complex profiles with a lot of peaks of both analytes and unknown substances. The best separation corresponded to that providing the highest number of peaks although the analysis time was also considered to be important. The elution gradient selected previously for standard separation was also convenient for sample extracts. As an example, Fig. 3 depicts a chromatogram of a pear extract in which peaks were separated with good resolution for most of the components.

Figures of merit

Figures of merit were assessed with DMSO synthetic standards according to FDA and Eurachem guidelines on validation of analytical methods for food analysis. Acceptance criteria of each analytical parameter were also defined as recommended elsewhere[55-57]. Linearity was evaluated at the selected wavelengths for each polyphenol as specified in the experimental section and Table 2. The method was linear within the range of concentrations assayed here, with regression coefficients r^2 better than 0.999 for most of the analytes (the acceptance criterion of linearity, r^2 should be > 0.995). The sensitivity of the calibration curve, expressed as AU × min × L × mg^{-1}, varied from 59.18 for *p*-coumaric acid to 4.34 for protocatechuic acid. Intra-day repeatabilities from 10 independent assays ($n = 10$) were, for retention times, better than 1.5%, lower than 0.5% for most of the components (acceptance criterion of time repeatability, *RSD* should be $<2\%$). For peak areas, the intra-day repeatabilities were about 2% (the acceptance criterion of peak areas, *RSD* should be $<10\%$ at the target concentration). In general, detection limits (LODs), stablished at a signal-to-noise ratio of 3, were below 0.2 mg L^{-1}. Additionally quantification limits (LOQs), stablished at a signal-to-noise ratio of 10, were below 0.6 mg L^{-1}. The specificity on synthetic standards was studied from the chromatographic resolution of close peak. The test was entirely satisfactory as, in any case, resolution values were better than 1.3.

Matrix effects were assessed from the comparison of calibration curves in DMSO and pear matrices. DMSO standards were prepared in the working range 0.5 to 20 µg mL^{-1} of each analyte to estimate the sensitivity in the absence of sample matrix. The same concentrations were added to pear extracts to run standard addition calibration curves. Results from various representative compounds, those

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more abundant in pear, are given in Table 3. In all these cases, the sensitivities were similar. This finding was generalized to the rest of compounds under study. The acceptance criterion (slope variability should be < ± 20%) was attained and matrix effects were considered to be negligible. As a result, calibration with DMSO standards was expected to be appropriate for the quantification of extractable polyphenols in pear.

The specificity was also tested in pear matrices. In this case, as samples may contain unknown components, this feature of the method was investigated from peak purity assays by comparing UV-vis spectra throughout each analyte peak. As spectral differences were irrelevant, it was concluded that the method specificity was satisfactory. The accuracy of the proposed method was estimated according to a spiking/recovery approach at a level 5 µg mL^{-1} of each analyte for n = 3 replicates. Quantitative recoveries corresponding to the ratio experimental/calculated concentrations were expressed as percentages. For most of the analytes recoveries were between 90 and 110% (see Table 3), so that the acceptance criterion of recovery was fulfilled (recoveries should be < ± 20%). In general, calibration models from DMSO standards provided acceptable quantifications that demonstrated the applicability of the proposed method to pear analysis.

Determination of polyphenols in pears

Pears of several varieties were analyzed according the proposed method. For each sample, three independent replicates were carried out. Some representative chromatograms are depicted in Fig 4. The profile of *alejandrina* is the most complex with multiple peaks in the range of phenolic acids and several signals of flavonoids. *Conference* and *blanquilla* also shows diverse peaks corresponding to the different families. The simplest chromatogram is attributed to *Williams* pears. In general, the diversity of polyphenols and their concentration ranges were very different depending on the varieties studied. It was found that some polyphenols were highly specific such as taxifolin and epigallocatechin which were characteristic of *conference* and *alejandrina*, respectively. Chlorogenic and neochlorogenic acids were common to all varieties with concentrations ranging from 0.003 to 0.03 mg g^{-1} and 0.002 to 0.005 mg g^{-1}, respectively.

From these studies it was concluded that there were qualitative and quantitative differences in the compositional profiles as a function of varieties. These results also suggest that some compounds might be potential quimiotaxonomical markers of pears to be exploited for characterization and authentication purposes.

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298 As a complementary study, the evolution of polyphenolic contents as a function of ripening time
299 was studied using pears of *conference* variety. A series of 11 pears belonging to the same set were let to
300 ripen for 23 days. Pears were analyzed, one by one, on days 1, 2, 5, 7, 9, 12, 16, 19, 21, 22 and 23 to
301 follow the evolution of amounts of components. In general, the behavior was highly depended on the
302 polyphenols considered. It was found that concentrations of some compounds decreased over time (e.g.
303 chlorogenic acid). In contrast, others species contents increased with time (e.g. gallic acid). Finally,
304 concentrations remained approximately constant for a few of components such as arbutin. This noticeable
305 effect of ripening on the compositional profiles was an issue to be taken into account when tackling the
306 determination of polyphenols. Despite such trends, the compositional characteristics of pears remain
307 approximately constant for 5 days. Hence, regarding analytical and nutritional concerns, the properties of
308 pears can be considered quite stable within this period of time when stored under refrigeration.
309

310 **Conclusions**

311 In this paper we established a simple, precise and accurate method for the determination of polyphenols in
312 pears. The optimization of both extraction and separation steps was based on experimental design in order
313 to find out efficient working conditions. This method enabled the identification and quantification of
314 several relevant compounds commonly found in pear pulp (such as arbutin and chlorogenic acid).
315 Furthermore, the comparison of chromatographic profiles of pears from different varieties revealed
316 important differences in their composition. It was found that some compounds were present in higher
317 proportions in some varieties while they were less important in others. Besides, specific compounds of
318 each a particular variety were encountered. As a conclusion, the present study could serve as a starting
319 point for future research to characterize, classify and verify the protected designation of origin (PDO) of
320 pears based on the compositional profiles associated to the polyphenolic fraction.
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7 399 **Figure 1.** Influence of the extraction solvent on the overall recovery of polyphenols in pears. Mean
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9 400 recovery considering several phenolic acids, alcohols and flavonoids (arbutin, gallic acid, chlorogenic
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11 401 acid, ferulic acid, coumaric acid, fisetin, quercetin and taxifolin).
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15 403 **Figure 2.** Chromatograms of the optimization of the elution gradient by experimental design. (a)
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17 404 Experimental design; (b) Chromatograms. Peak assignment: 1: arbutin, 2: gallic acid, 3: protocatechuic
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19 405 acid, 4: protocatechuic aldehyde, 5: tyrosol, 6: catechin, 7: chlorogenic acid, 8: 4-o-cafeolquinic acid, 9:
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21 406 vanillic acid, 10: caffeic acid, 11: epicatechin, 12: syringic acid, 13: ethyl gallate, 14: coumaric acid, 15:
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23 407 taxifolin, 16: ferulic acid, 17: sinapinic acid, 18: resveratrol.
24
25 408
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27 409 **Figure 3.** Chromatogram of a pear extract obtained under the optimal extraction and separation
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29 410 conditions.
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31 411
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33 412 **Figure 4.** Representative chromatograms of the pear varieties. (a) *Alejandrina*; (b) *Blanquilla*; (c)
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35 413 *Conference*; (d) *Ercolini*; (e) *Williams*. Peak assignment: 1: arbutin, 2: gallic acid, 3: protocatechuic acid,
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37 414 4: neochlorogenic acid, 5: procyanidin B1, 6: protocatechuic aldehyde, 7: tyrosol, 8: 4-hydroxybenzoic
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39 415 acid, 9: chlorogenic acid, 10: 4-o-cafeolquinic acid, 11: caffeic acid, 12: epicatechin, 13: syringic acid,
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41 416 14: syringic aldehyde, 15: epigallocatechin 16: coumaric acid, 17: ferulic acid, 18: sinapinic acid, 19:
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43 417 quercetin-3-galactoside, 20: rutin, 21: quercitrin, 22: fisetin, 23: kaempherol, (*) gradient peaks.
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3 421 Table 1. Extraction Recoveries Estimated from the Comparison of Peak Areas of
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5 422 Analyte Additions Pre- and Post-Extraction
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Family	*Model polyphenol*	*Extraction Recovery (%)*
Benzoic acids	Gallic	73 ± 2
	p-Hydroxybenzoic	84 ± 3
	Syringic	85 ± 1
Cinnamic acids	Chlorogenic	79 ± 3
	Neochlorogenic	78± 3
	Caffeic	80 ± 2
Phenyl alcohols	Arbutin	79 ± 8
Stilbenes	Resveratrol	81 ± 4
Condensed tannins	Procyanidin B1	60 ± 8
Flavanols	Epicatechin	65 ± 8
Flavonols	Quercetin	62 ± 5
	Quercetin-3-glucoside	70 ± 3
Flavones	Apigenin	70 ± 3
	Rutin	70 ± 3

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425 Table 2. Figures of Merit of the Method for Some Polyphenols

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Family	*Model polyphenol*	*λ (nm)*	*Repeatability Time*	*Repeatability Area*	*Sensitivity*	r^2	*LOD (mg mL^{-1})*	*Quantitative Recovery (%)*
Benzoic acids	Gallic	280	2.4	2.8	24.3	0.997	0.11	90 ± 8
	p-Hydroxybenzoic	280	1.1	0.7	17.5	0.9999	0.04	90 ± 6
	Syringic	280	1.6	0.7	29.8	0.9997	0.10	85 ± 6
Cinnamic acids	Chlorogenic	310	0.5	2.6	12.8	0.999	0.28	102 ± 2
	Neochlorogenic	310	1.1	2.3	10.4	0.998	0.04	97 ± 7
	Caffeic	310	1.3	2.2	23.7	0.9998	0.30	93 ± 6
Phenyl alcohols	Arbutin	280	0.5	2.7	3.7	0.9994	0.13	100 ± 3
Stilbenes	Resveratrol	280	2.3	1.0	33.7	0.9999	0.11	97 ± 3
Condensed tannins	Procyanidin B1	280	0.7	4.8	3.0	0.9999	0.16	117 ± 2
Flavanols	Epicatechin	280	2.8	2.5	7.3	0.996	0.13	90 ± 5
Flavonols	Quercetin	370	1.9	1.5	10.3	0.999	0.21	99 ± 5
	Quercetin-3-glucoside	370	2.0	3.6	9.9	0.998	0.14	96 ± 5
Flavones	Apigenin	370	1.7	3.0	27.7	0.999	0.16	99 ± 5
	Rutin	370	2.9	1.8	8.2	0.9992	0.20	97 ± 5

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Table 3. Evaluation of Matrix Effects. Comparison of Slopes in DMSO (b1) and Pear Extracts (b2). Ratio b1/b2 Expressed as a Percentage

Family	***Model polyphenol***	***b1/b2 (%)***
Benzoic acids	Gallic	90
	p-Hydroxybenzoic	108
Cinnamic acids	Chlorogenic	92
	Caffeic	106
Phenyl alcohols	Arbutin	96
Stilbenes	Resveratrol	94
Flavanols	Epicatechin	100
Flavonols	Quercetin	106
	Quercetin-3-glucoside	105
Flavones	Apigenin	85
	Rutin	98

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434 **Figure 1**

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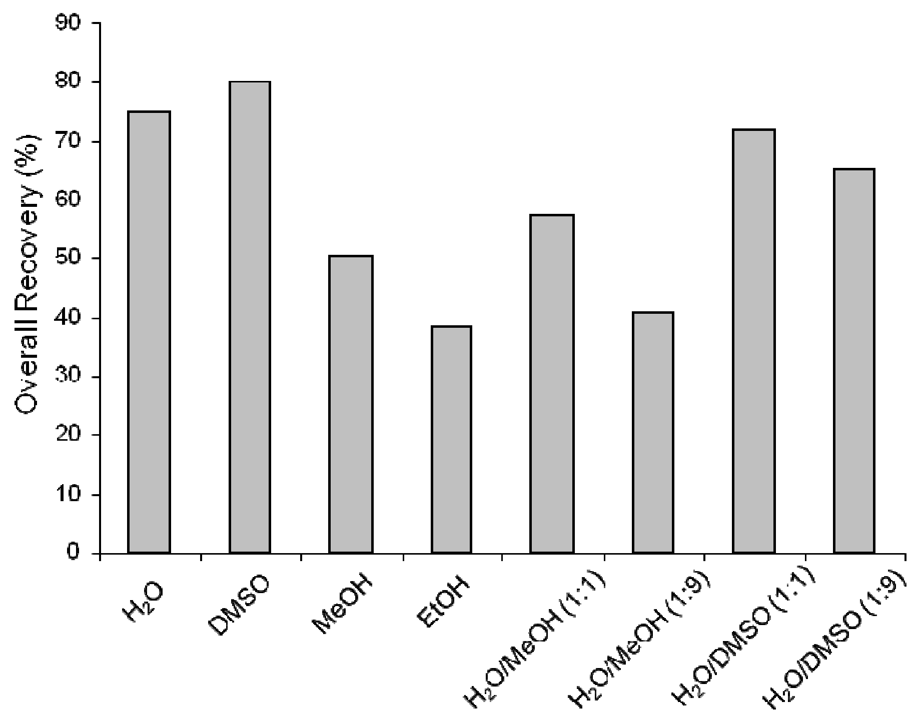

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Figure 2

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442 **Figure 3**

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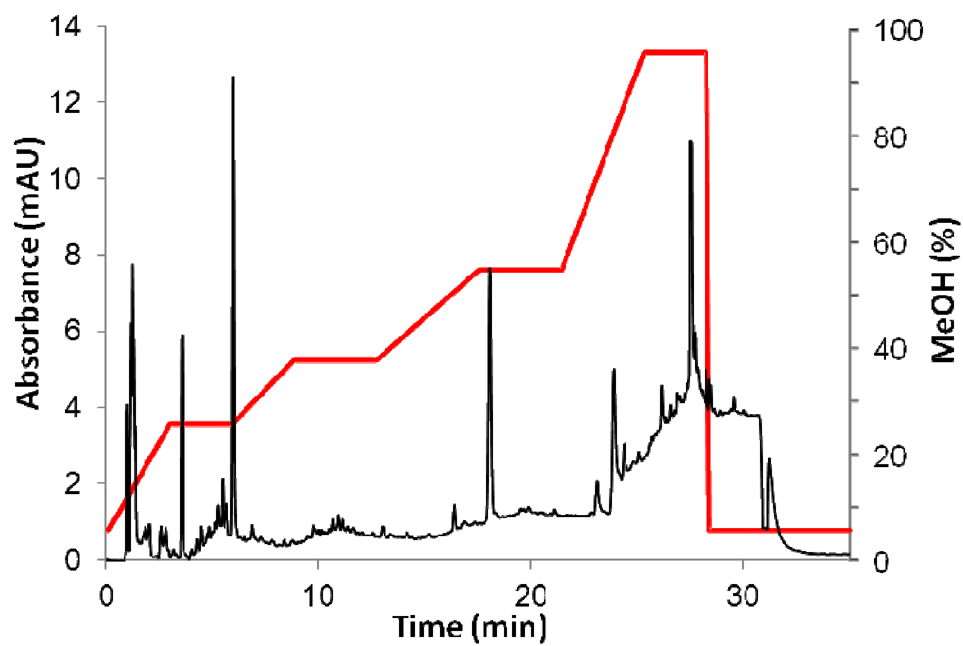

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1 446 **Figure 4**
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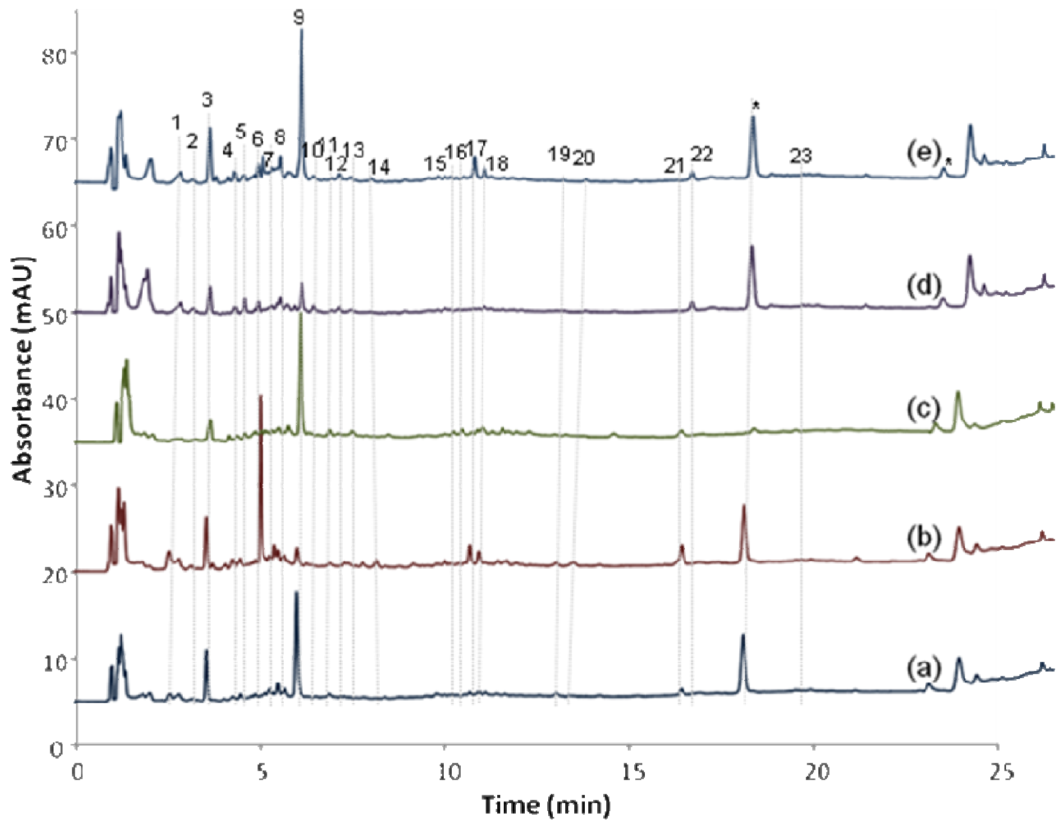

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Graphical abstract

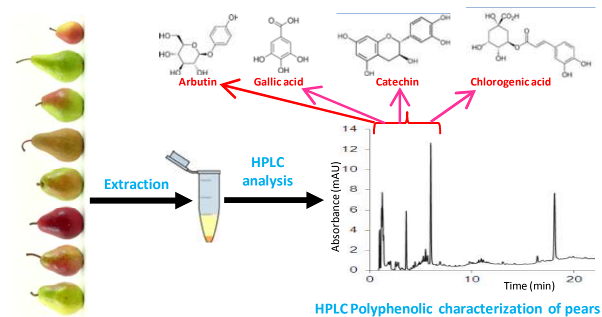

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Figure 1

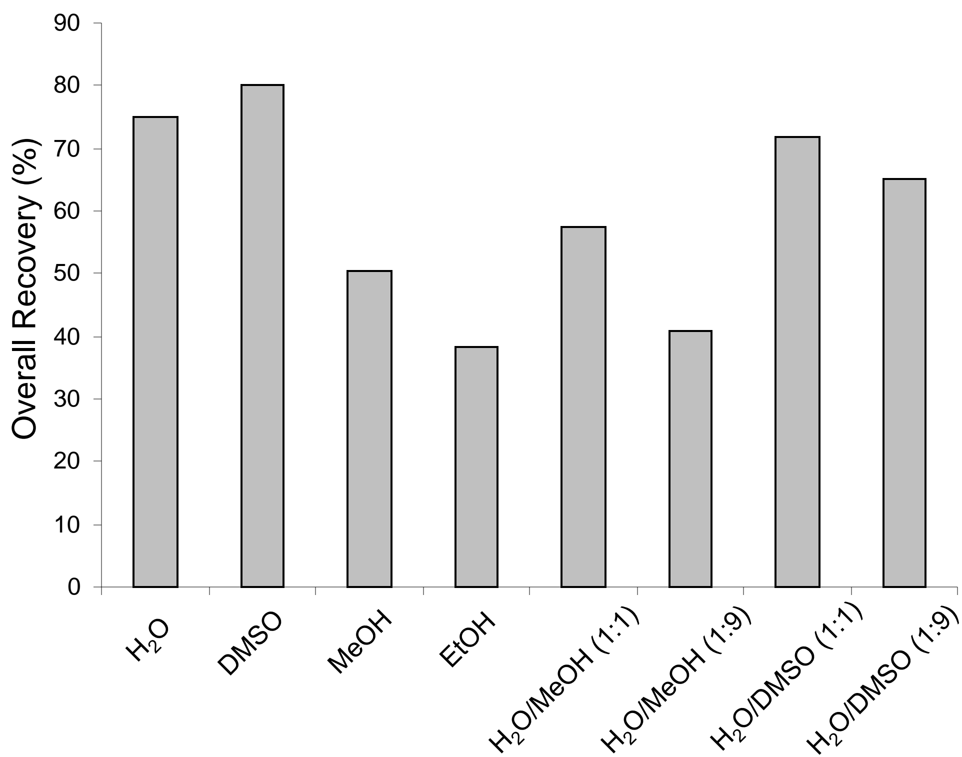

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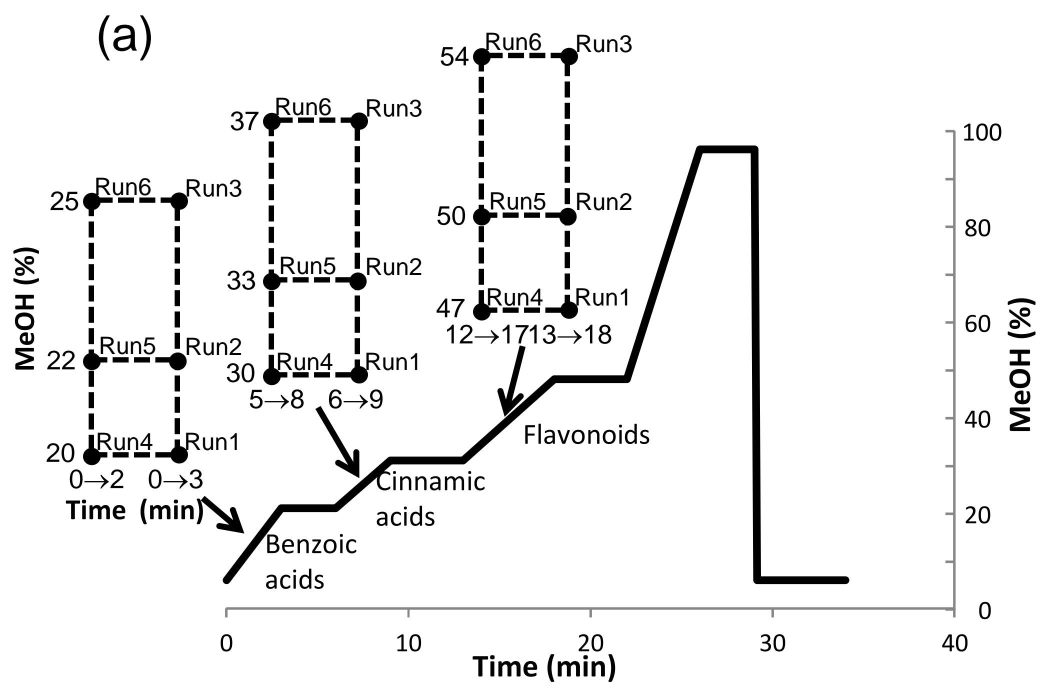
Figure 2



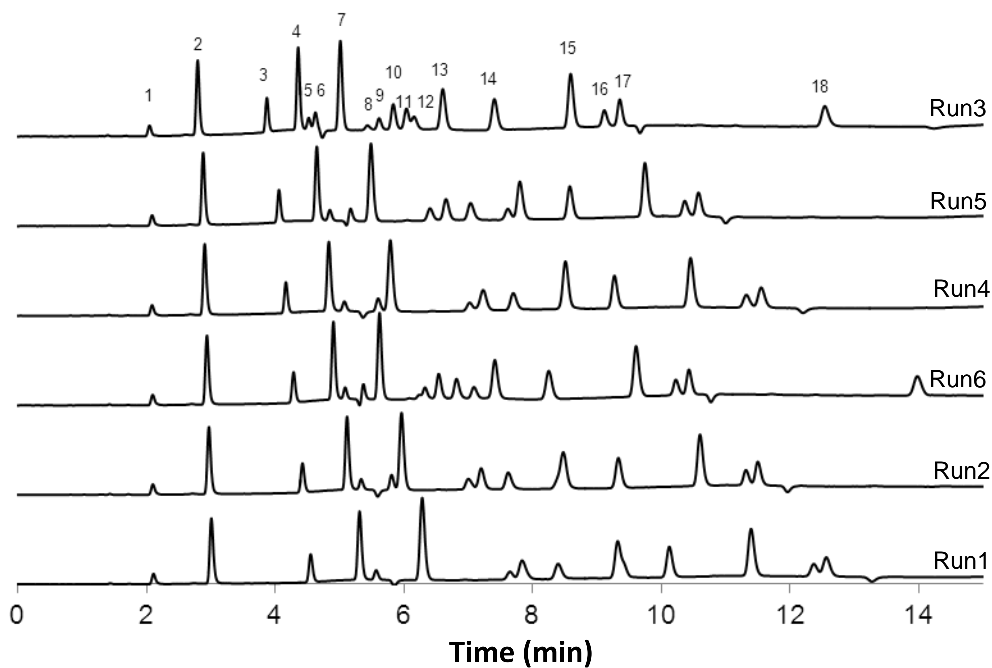


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Figure 3

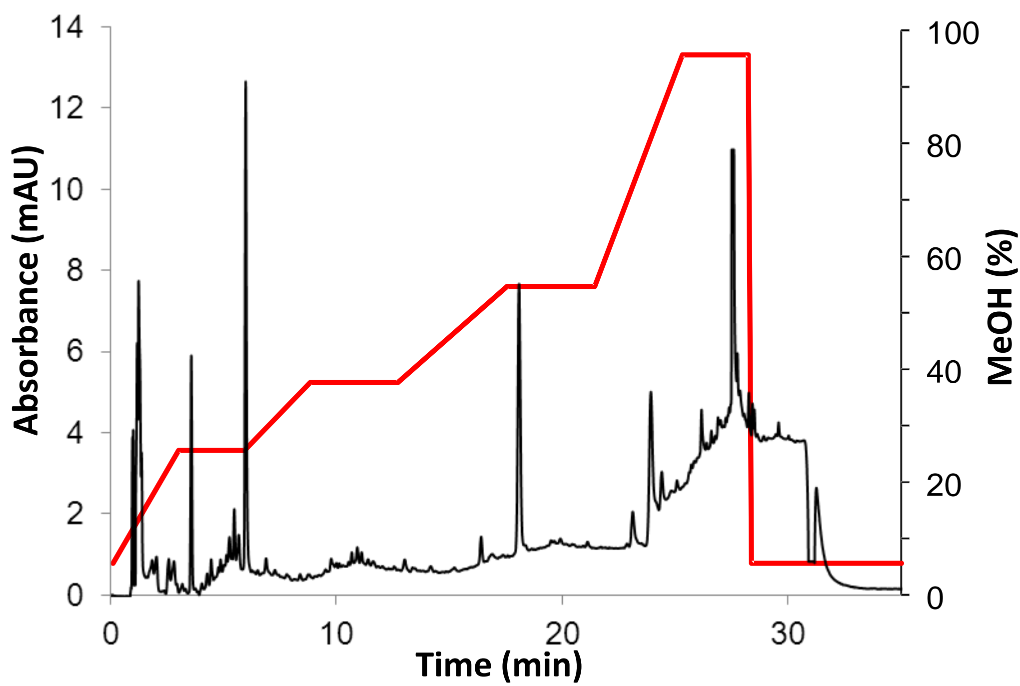

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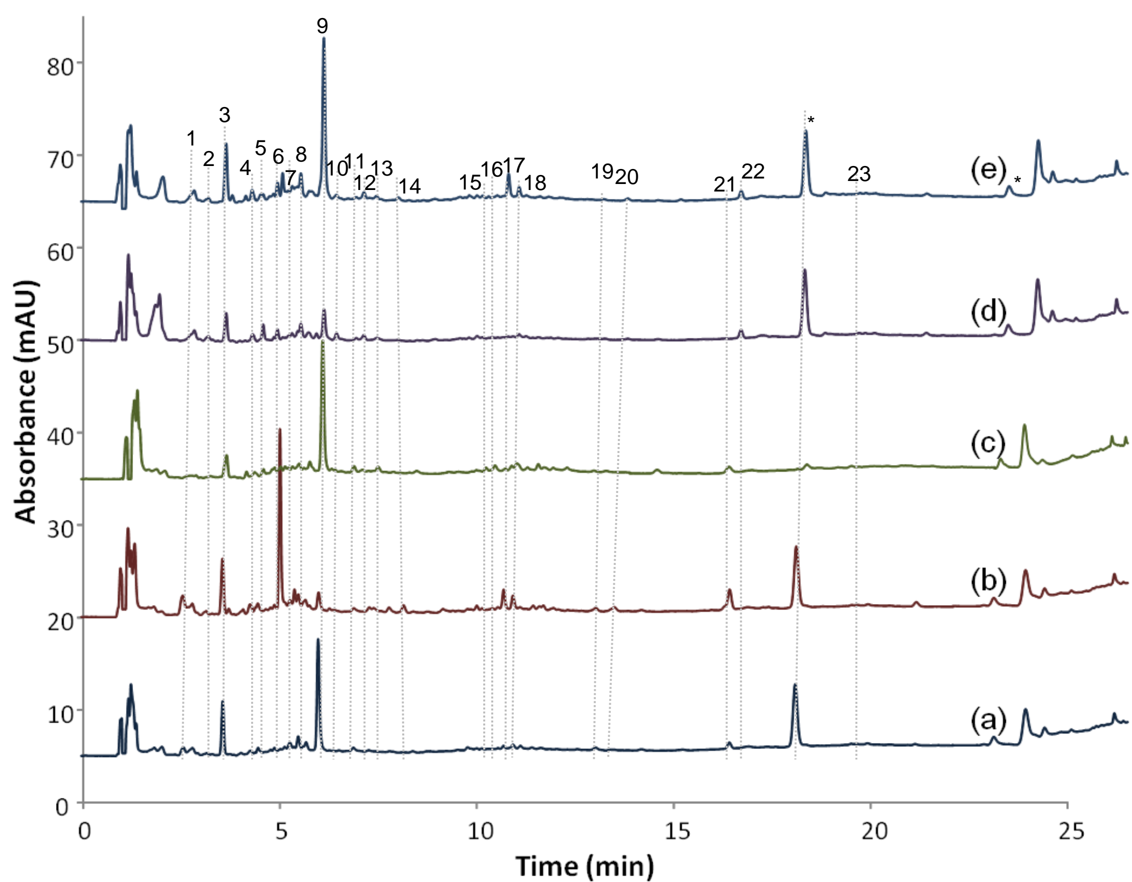
Figure 4



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