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Abstract. A new chromatographic method by Ultra High Performance liquid chromatographic (UHPLC) technology, has been developed and validated for the determination of polydatin and resveratrol, as potential metabolite, in human plasma. After the optimization of the chromatographic conditions, the method has been validated on spiked human plasma samples. The optimized extraction allowed to obtain analytes recovery up to 98,48 ± 4,03%. Then, the isocratic elution in reversed phase mode, provides the separation of polydatin and resveratrol in less than 10.0 min. Chromatographic analysis was performed on a C18, 10cm x 3.0mm, 2.7 µm stationary phase, by using triethanolamine phosphate solution (0.1 M, pH= 3.7) and ACN 85:15 (v/v) as mobile phase at a flow rate of 0.5mL/min. The UV detector was set at 306 nm for the analysis of both polydatin and resveratrol. The limit of detection (LoD) and the limit of quantification (LoQ) for polydatin in plasma samples were found to be 7,82±0,38 nM and 26,06±1,28 nM respectively. The method was found to be accurate and precise with a coefficient for intra- and inter-day variation below 5%. All the reported data demonstrate how the developed method is rapid and sensitive. Moreover, results of the analysis of plasma samples, obtained from orally treated volunteers with nutritional supplements containing polydatin, have shown the method to be suitable for the pharmacokinetic characterization of polydatin and resveratrol, as metabolite, in humans.

KEY WORDS: Polydatin, resveratrol, Ultra High Performance Liquid Chromatographic analysis, human plasma, pharmacokinetics.

1. Introduction

3,4,5-Trihydroxystilbene-3-beta-monoglucoside or resveratrol 3-beta-mono-D-glucoside (polydatin) is a natural precursor of resveratrol (3,4,5-Trihydroxystilbene). Polydatin is the product of resveratrol combined with glucose (Fig. 1). Both resveratrol and polydatin are extracted from the radix or rhizome of *Polygonum cuspidatum* used in Japanese and Chinese folk medicine for the treatment of some cardiac ailments, including inflammation, atherosclerosis and neuropsychiatric disorders[1] [2]. Stilbenes are also present in several foods as peanuts, blueberries, bilberries, cranberries and grapes [3]. Interestingly, polydatin was found to be the major component in grape juices [4] and it is more abundant than resveratrol in nature [5].

The interest in these molecules has to be ascribed to their wide range of properties. Many studies were published depicting the various and different effects of resveratrol [6-9], especially those related to chemoprevention, cardiovascular diseases, aging and cancer [10, 11]. At the same time also polydatin demonstrated properties similar to those of resveratrol in inhibiting platelet aggregation [12], in inhibiting oxidation of human LDL [13], in reducing eicosanoid synthesis [14], in reducing the elevations of lipid levels [15] and in obtaining anti-carcinogenesis effects [16]. Among the so many pharmacological benefits of both stilbenes, the antioxidant one is undoubtedly the most relevant. Polydatin alone or in combination with resveratrol, modulates oxidative stress by affecting the mitochondrial superoxide anions, extracellular NO production, and the scavenger enzymes [17]. That is exactly why both molecules have received increasing attention.

Furthermore, polydatin was demonstrated to have higher scavenging activity against hydroxyl radicals than resveratrol both *in vitro* [18] and *in vivo* [19]. The major effects exerted by polydatin can be ascribed to its higher oral bioavailability. Different studies were carried out to explore metabolism of resveratrol and polydatin *in vivo*. The results revealed resveratrol to be subjected to a rapid and extensive metabolism with the consequent formation of different metabolites as resveratrol-sulfate and resveratrol-glucuronides [20, 21], and polydatin to convert into resveratrol [22, 23]. In particular, studies depicting the pharmacokinetic profile of polydatin in rats indicates it undergoes extensive

first-pass deglycosylation and glucoronidation. Therefore, polydatin is primarily metabolized to resveratrol in the small intestine and liver. Then resveratrol is then metabolized into glucoronidated form. Resveratrol and glucoronidated resveratrol are mainly excreted by the kidneys [23, 24].

In light of these evidence, resveratrol might be substituted by polydatin in clinical use. All this evidence led to the blossom up of many botanical dietary supplements containing polydatin. This implies the need of developing methods for the pharmacokinetic study of these active components, in order to check the posology and the elucidation of polydatin metabolism rate and its metabolites. As well as pharmaceuticals, botanical dietary supplements, should in fact be developed to ensure a safe and effective product. The steps to follow include the characterization of absorption, metabolism and bioavailability of active compounds [25]. In this context, many studies were based on the detection of polydatin, resveratrol and their metabolites in rat plasma [23, 24, 26-29], since *in vivo* animal studies are used prior to clinical studies. The metabolic profile of resveratrol was also monitored in healthy humans after consumption of red wine and resveratrol tablets [30-32]. All these studies were carried out by applying high performance liquid chromatography (HPLC) coupled to UV or mass spectrometry detection systems.

Since no studies were carried out for the characterization of polydatin pharmacokinetic in humans so far, we developed a highly sensitive, selective and fast Ultra High Performance liquid chromatographic (UHPLC) method for the determination of polydatin and resveratrol, as potential metabolite, in human plasma. The new method was applied to monitor polydatin distribution after dietary supplements (containing 40 or 75 mg of polydatin) administration to 6 healthy volunteers, by analyzing blood samples from all volunteers for 1 months. One urine sample was also analysed by employing the same UHPLC method.

2. Material and methods

2.1 Materials

Triethanolamine, phosphoric acid, Methanol and acetonitrile of HPLC grade, were obtained from Sigma Aldrich (St. Luis, MO, USA). Polydatin and resveratrol (purity ≥99,0%) and nutraceutical tablets

containing 40 and 75 mg of polydatin were kindly provided by Ghimas S.p.A., Bologna Italy. Water of ultrapure grade was purchased by a Merck Millipore system (Burlington, MA, USA). Human plasma and urine were obtained from healthy volunteers in agreement with local ethics guidelines.

2.2 Standard solutions

Both polydatin and resveratrol were accurately weighted and dissolved in methanol in order to obtain 1mg mL^{-1} stock solutions. The obtained solutions were stored at -20°C and maintained protected from light. The solutions were found to be stable for more than one month.

2.3 Chromatographic method optimization

The UHPLC-DAD analyses were carried out by using a Jasco X-LC (Jasco Europe, Cremella, Italy), consisting of a binary pump (3185PU), autosampler (3059AS), a temperature controller column compartment (3067C0), a degasser module (3080DG) and a Detector X-LC^{TM} 3110 MD diode array (DAD). Instrument control, data acquisition and processing were performed with software CromNAV Control Center. A reversed phase column (Sulpeco, Ascentis® Express (Bellefonte, PA, USA) C18, 10cm x 3.0mm, 2.7µm) was equilibrated with a mobile phase consisting of triethanolammonium phosphate (0.1 M, pH= 3.7) and acetonitrile 85:15 (v/v). The column temperature was kept at 40°C. The UV detection wavelength was set at 306nm. Optimized chromatographic separation of polydatin and resveratrol was carried out under isocratic elution at a flow rate of 0.5 mL min^{-1} with total runtime of 14 minutes.

The resolution between sequential peak pairs was calculated applying the equation reported below: $Rs=2(t_{R2}-t_{R1})/(W1+W2)$.

Besides retention time, analyte identification was also performed by comparing online UV spectra acquired on standards peak apex with those from sample analysis.

2.4 UHPLC method validation

The proposed analytical method was validated in terms of linearity, specificity, sensitivity and accuracy.

The linearity of the new method was determined on spiked plasma samples obtained by adding 5µL of analyte solution to 45µL of plasma from non-treated volunteers. According to the sensitivity of the method, the final stilbene concentrations obtained were comprised in the range 0.30-5 µM. Protein precipitation and analyte extractions were carried out by adding 150 µL of MeOH to the spiked plasma. Samples were stirred for 10 s before being centrifuged for 10 min at 9600 x g at 4°C. Then the supernatant was collected, and the solvent removed under nitrogen flux overnight. The solid was dissolved in 100µL of mobile phase and it was centrifuged at 9600 x g for 10 minutes at 4 °C. An aliquot of 5µL of supernatant were then injected into UHPLC-DAD system. The experiments were performed in triplicate. Calibration curves were then obtained by linear least-squares regression analysis by plotting peak area versus analytes concentration.

The specificity of the method was determined by using three human blank serum plasma samples by comparing the chromatograms obtained after injection of non-spiked and spiked blank plasma samples respectively. Plasma samples, spiked with standard mixtures of polydatin and resveratrol, respectively, in order to obtain 0,31 µM; 1,25 µM and 5,00 µM final concentrations, were subjected to extraction and analyzed by UHPLC according to the method reported in 2.3 paragraph.

The LoD (S/N=3) and LoQ, (S/N=10) were determined by performing UHPLC-DAD analysis in triplicate of incremental dilutions of stilbenes solutions obtained by methanol extraction from spiked plasma. Then the accuracy was determined by calculating the percentage of the deviation between the nominal and the experimental concentrations of polydatin and resveratrol obtained from plasma analysis for the three different concentrations. The intra- and inter-day precision were estimated by analyzing samples enriched with both the stilbenes at low (0,31µM) medium (1,25 µM) and high (5,00 µM) concentrations during the same day (n=5) and in different days (n=10).

2.5 Stability determination

A number of 8 plasma samples spiked with polydatin solution to the final concentration of 2 µM, were stored at RT for 10min, 20min, 30min, 40min, 60min, 120min, 180min and 240min before being subjected to extraction. The obtained solutions were then analyzed by UHPLC.

2.6 Sample analysis

The validated method was applied to the determination of polydatin in human plasma of 6 healthy volunteers. For 5 of them, bloody samples were obtained at 1, 2 and 3 hours after the first oral administration of 40 mg polydatin tablet, and after one month of treatment. Only for one of the six volunteers the blood samples were obtained after 1, 2 and 3 hours after the first oral administration of 75 mg polydatin tablet. For this same volunteer both blood and urine samples were collected after one month of treatment.

The blood obtained from volunteers was collected into anticoagulant-treated tubes. Cells were removed from plasma by centrifugation for 15 min at 95 x g at 4°C. Plasma was then divided into 0.30 mL aliquots in polypropylene tubes and kept at -80°C.

The standard addition method (SAM) was then applied to determine the concentration of polydatin in human matrix. Each sample has been spiked with various amount of polydatin standard solution in order to achieve the known final concentrations of 0,15 µM; 0,30 µM and 0,45 µM.

A 1:3 methanol extraction has been carried out for each sample. The samples were vortexed for 10 seconds after solvent addition and centrifuged at 9600 x g for 10 minutes at 4 °C. The supernatant was collected, and the solvent was removed under nitrogen flux overnight. The solid was then dissolved in 100µL of mobile phase and it was centrifuged at 9600 x g for 10 minutes at 4 °C before injection in the UHPLC.

For what concerns the urine sample, 150 µL of acetonitrile were added directly to 850µL of urine sample. Then, the resulting mixture was centrifuged for 10 min at 9600 x g at 4°C (Micro Star 17R, VWR centrifuge), the supernatant was collected and analyzed by the developed UHPLC method.

3. Results and Discussion

Several nutritional supplements containing polydatin have been launched on the marked in the last few years due to the excellent antioxidant properties of this stilbene and its improved bioavailability. Therefore, the development of an analytical method for the pharmacokinetic characterization of polydatin results of fundamental importance for establishing absorption, distribution metabolism and excretion (ADME) parameters for dietary supplements before being launched on market [25]. In

this context, the aim of this work was to develop a chromatographic method with DAD detector for the determination of polydatin and resveratrol, as metabolite, in human plasma. As suggested by studies on rats, polydatin is indeed metabolized primarily to resveratrol in the small intestine and liver. Then resveratrol and its metabolized glucoronidated form are in turn mainly excreted by the kidneys [23, 24].

The developed method was first validated on solutions of polydatin and resveratrol obtained after extraction carried out on spiked plasma samples obtained from three healthy volunteers. The analysis of chromatograms obtained for the samples containing fixed amounts of stilbenes allowed to determine linearity, sensitivity, accuracy and precision of the new method.

3.1 UHPLC method optimization

The chromatographic conditions were optimized on the basis of selectivity, peak resolutions, baseline drift and elution time. Investigated variables were pH of the mobile phase buffer (pH range from 3.7 to 7.00), type of buffer salts (triethylammonium phosphate, sodium phosphate), buffer concentration (from 0.05 to 0.30), percentage (from 5 to 40%) and type (methanol, acetonitrile) of organic modifier and type of reversed phase stationary phase (C8 and C18). The preliminary analysis were performed by a HPLC-DAD Agilent (Santa Clara, CA, USA) 1260 Infinity LC System and performed on a Zorbax Eclipse Plus Agilent column (Santa Clara, CA, USA) C8 2.1X100 3.5 µm**.** However, the optimised conditions were obtained on a C18, 10cm x 3.0mm, 2.7 µm stationary phase, by using triethanolamine phosphate solution (0.1 M, pH= 3.7) and ACN 85:15 (v/v) as mobile phase at a flow rate of 0.5mL/min. For the analysis of both polydatin and resveratrol the UV detector was set at 306 nm, which is the maximum wavelength in the UV-Vis spectrum obtained online. In the mobile phase acetonitrile decreased viscosity and backpressure when compared with methanol and the most suitable percentage for the organic modifier was found to be equal to 15%. In fact, the use of lower amount of ACN (10%) increased the retention time of both the analytes with run time analysis longer than 20 min. On the other hand, the use of a higher percentage of organic modifier (20%) did not guarantee the required selectivity. A pH value equal to 3.7 for mobile phase was found optimal to obtain a good resolution for both the analytes (Rs>20), when compared to a neutral pH, by improving efficiency, due their slightly acidic character of the phenols groups of the analytes. The

triethanolammine added in buffer further contributed to decreasing the analytes secondary interactions with free sylanols of the stationary phase, avoiding peak narrowing. Moreover, all these conditions used in an UHPLC system, on a 2.7 µm particle size C18 column guaranteed even better analysis performances. Indeed, the use of smaller particle size column resulted in an increased efficiency and as consequence in higher resolution and sensitivity.

3.2 Sample preparation

The optimal conditions for plasma extraction were achieved, according to the protocol reported by Lv and co-worker in 2006 [35], for the determination of polydatin in plasma and tissue rat samples. We adopted methanol as solvent for protein precipitation at a plasma to solvent ratio of 1:3 [27]. The use of methanol for plasma extraction is widely used in most of the protocols adopted for protein precipitation and extraction of metabolites. The most common plasma to solvent ratio is reported to be 1 to 3 or 4 [33-35]. Acetonitrile, methanol and mobile phase were also tested for sample reconstitution after solvent evaporation under nitrogen flux. The use of the mobile phase, provided higher recovery values.

3.3 UHPLC method validation

Before starting to analyze plasma samples, the solutions obtained after methanol extraction of blank plasma, and of plasma spiked with standards of polydatin and resveratrol, were analyzed in order to verify the absence of any interferences (Fig. 2). Both the analytes show a typical UV absorption band with a maximum wavelength at 306nm, endowed of a certain degree of selectivity [19]. So, the absence of interferences detected at 306nm confirmed the specificity of the method.

The linearity and the sensitivity of the method were determined. Calibration curves were obtained for polydatin and resveratrol by performing UHPLC-DAD analysis in triplicate on plasma samples, spiked with standard solutions of stilbenes in order to obtain five incremental concentrations (0.31 µM; 0.63 µM; 1.25 µM; 2.50 µM and 5.00 µM), after methanol extraction procedure, evaporation and mobile phase residue solubilization. A satisfactory correlation coefficient (r^2>0.996) was obtained

for the calibration curves of both the stilbenes. The LoQ and LoD were also determined for both the analytes by using solutions obtained after extraction carried out on plasma spiked with standards. The LoD and LoQ values for polydatin were found to be equal to 7.82±0,38 nM and 26.06±1.28 nM respectively. For resveratrol a LoD value of 76.28±8,57nM and a LoQ value of 254.26±28.57 nM was found. All the obtained results are reported in table 1. As discussed in the previous paragraph the use of 2.7 µm particles stationary phase in an UHPLC equipment has led to an improvement in efficiency and therefore sensitivity of the proposed method compared with that obtained performing traditional HPLC analysis in the same chromatographic conditions. The LoQ and LoD values for polydatin found by applying the traditional HPLC approach were found to be three times higher than those calculated by UHPLC method. Moreover, the injection volume for the HPLC application was 4 times larger than that required for the proposed UHPLC method. The retention times of stilbenes was improved too, since the analysis time in a HPLC apparatus was almost doubled in the same condition (10 minutes run time versus twenty minutes).

To verify both accuracy and precision, a recovery test was carried out on blank plasma samples, from three different healthy volunteers, spiked with three incremental dilutions of the two stilbenes in order to obtain the final concentrations of 0.31; 1.25 and 5.00 µM. The analysis were carried out in duplicate for each plasma sample, by applying the described extraction method (par. 2.6). Then the accuracy was determined for the three different concentrations by calculating the percentage of the deviation between the nominal concentrations of polydatin and resveratrol and the experimental ones obtained from plasma analysis. The coefficient of variation (CV) of intra- and inter-day assays, performed analyzing in duplicate 10 and 5 different solutions respectively, were 1,93% and 5,83% for polydatin and 5,48% and 2,93% for resveratrol. The values for the inter-day and the intra-day precision and accuracy are shown in Table 2.

For what concerns the recovery, the mean value for this parameter was 98,48 ± 4,03% for both stilbenes at different tested concentrations (Table 3). The high level of recovery obtained confirmed that methanol is an optimal solvent for polydatin extraction, due to its high capability to precipitate proteins and analytes solubility properties. Moreover, the use of mobile phase for the reconstitution of extracts after solvent evaporation, guarantees the highest recovery when compared with methanol or acetonitrile (data not shown).

3.4 *Stability determination*

Polydatin stability was also determined by analyzing solutions obtained by methanol extraction carried out on spiked plasma samples, stored at RT, over time ranging from 10 to 240 min. The comparable peak areas obtained after each chromatographic analysis confirmed the stability of polydatin in plasma under the investigated conditions.

3.5 Sample analysis

The developed chromatographic method was applied to the determination of polydatin and eventually of resveratrol as its metabolite, in human plasma. The blood samples obtained from 6 different volunteers were collected at 1, 2 and 3 hours after the oral uptake of polydatin tablets and after one month of daily treatment. Only for one volunteer the treatment consisted in the uptake of a higher dosage of polydatin (75mg), while for the other volunteers the dosage was equal to 40mg. The chromatographic analysis of samples obtained from volunteers at increasing times revealed the presence of polydatin only in two plasma samples. The unknown polydatin concentration was then calculated by Standard Addition Method (SAM). Calibration lines were obtained by plotting the known analyte concentrations, added to the samples, versus the corresponding chromatographic peaks area. The concentration of polydatin in human matrix was then calculated from intercept with x-axis.

A concentration of polydatin equal to 10,06±0,22nM was found in plasma sample obtained from one of the five volunteers, treated with 40mg tablet, after one month of treatment. On the other hand, for the volunteer treated with the higher dosage of stilbene, the presence of polydatin at a concentration equal to 65,07±6,81nM µM was detected in the sample collected two hours after the uptake of 75mg tablet (Fig 3). These results led us to hypothesize that polydatin is very fast metabolized presumably to resveratrol in the small intestine and liver, which then is fast metabolized into glucoronidated form.

According to the obtained results and the evidence reported in literature for polydatin metabolism [23, 24], we also decided to analyze the urine sample of the volunteer treated for a month with the

higher dosage of stilbene, at one hour from the tablet uptake, in order to detect excreted analytes. Indeed, polydatin at a concentration equal to 531,82±3,59nM was determined. Moreover, the chromatographic analysis of the urine sample revealed the presence of potential metabolites, showing similar absorption properties but different retention times, whose UV spectra was found to be superimposable to the investigated analytes (lambda max 306nm), suggesting the presence of polydatin metabolites (Fig. 4). This preliminary result will be the object of further investigation specifically addressed to the characterization of polydatin metabolites in human matrices.

4. Conclusions and perspectives

An accurate, sensitive and selective UHPLC-DAD method was developed for the detection and quantification of polydatin in human plasma for the first time. The high reproducibility and recovery achieved for both the stilbenes allow the accurate detection of analytes at a concentration down to 7,82±0,38 nM for polydatin and 76,28±8,57nM for resveratrol. The method was found to be suitable for the detection of polydatin in human plasma and, in addition, in urine after oral administration of nutritional supplements. Therefore, it can be applied for the characterization of polydatin pharmacokinetic profile in humans. However, we think that a dose higher than 75 mg/day has to be administered in order to determine it at a concentration in the blood suitable to exert its properties. Moreover, due to its high first passage metabolism and according to the preliminary results obtained by analyzing urine sample from one treated volunteer with 75 mg tablets, we aim to investigate and characterize polydatin metabolites in urine of human volunteers in future studies.

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Analyte	Calibration Equation	r^2	LoD (nM in plasma)	LoQ (nM in plasma)
Polydatin	y=(2355±222,33)x - (184,47±53,48)	0.9967± 0,0016	7,82±0,38	26,06±1,28
Resveratrol	y=(2185±43,13)x + (52,07±27,56)	0.9964± 0,0016	76,28±8,57	254,26±28,57

Table 1. Linearity and sensitivity parameters for the detection of polydatin and resveratrol in human plasma by UHPLC-UV method.

	Nominal concentration (µM)	Measured concentration (µM)		Precision (RSD%)		Accuracy (relative error %)	
		Polydatin	Resveratrol	Polydatin	Resveratrol	Polydatin	Resveratrol
Intra-day (n=10)	0,31	0,31 ± 0,01	0,30± 0,01	1,64	1,03	-0,32	-4,69
	1,25	1,22 ± 0,01	1,25± 0,07	0,86	5,48	-2,65	-0,28
	5,00	5,00 ± 0,10	5,01± 0,08	1,93	1,50	-0,10	0,11
Inter-day (n=5)	0,31	0,33 ± 0,02	0,31± 0,01	5,83	0,93	4,01	0,73
	1,25	1,21 ± 0,01	1,24± 0,04	0,43	2,93	-3,13	-1,12
	5,00	4,91 ± 0,01	4,77± 0,11	0,19	2,34	-1,76	-4,83

Table 2. Inter- and intraday accuracy and precision for the determination of polydatin and resveratrol in human serum plasma. The analyses were carried out 5 times on the same day and 10 times over two consecutive days.

	Concentration µM	Recovery %	RSD%
Polydatin	0,31	94,80	3,92
	1,25	93,37	3,16
	5,00	97,43	2,00
Resveratrol	0,31	100,65	5,03
	1,25	104,07	2,84
	5,00	100,57	3,46

Table 3. Recovery values obtained for spiked plasma samples enriched with polydatin or resveratrol at different concentrations. The applied formula was: AE%= (B/A) x100 where A is the peak area obtained for standard solutions and B is the peak area of extracted plasma samples.

Legend of the figures

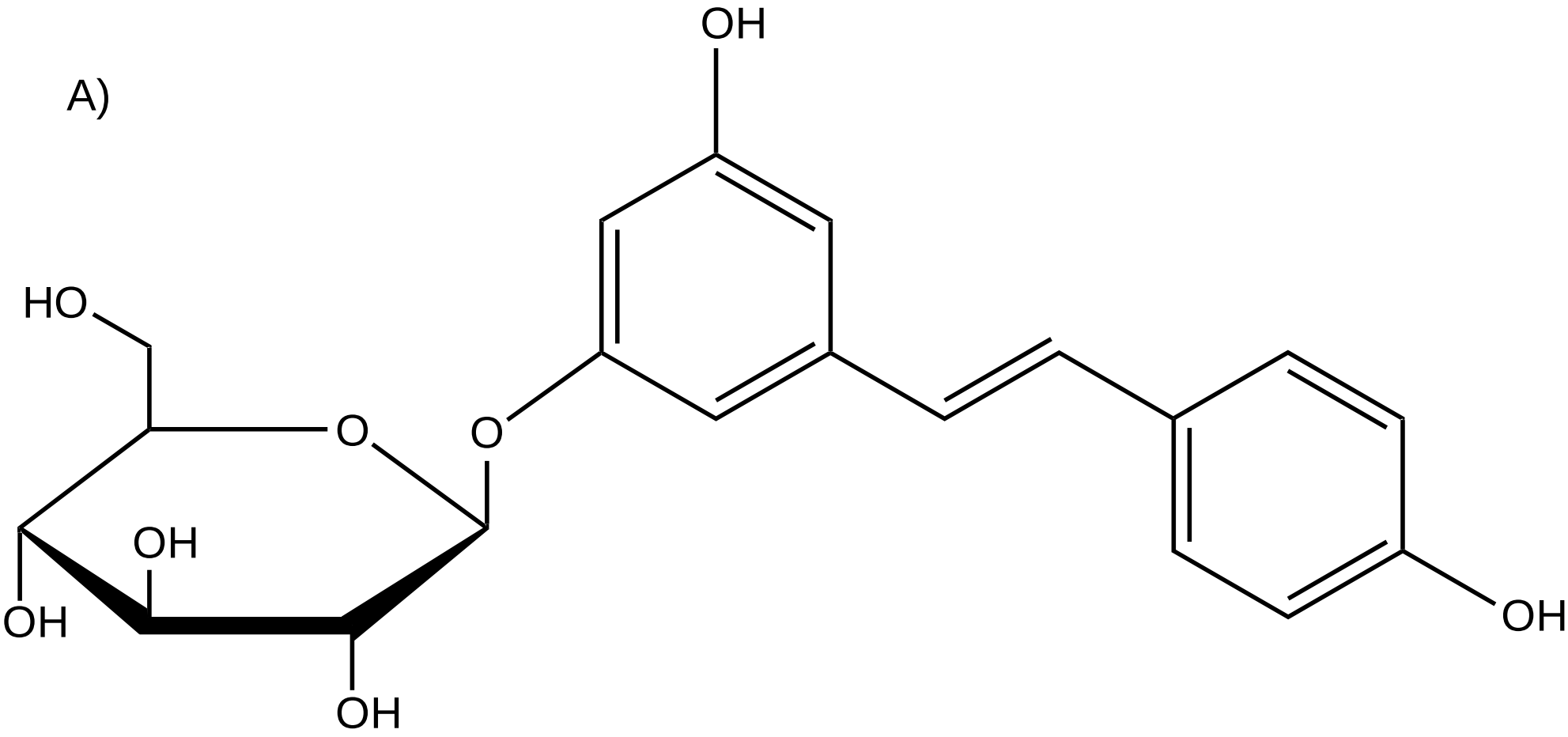
Fig. 1 Chemical structure of polydatin (A) and resveratrol (B)

Fig. 2 UHPLC chromatograms of blank serum sample (dotted line); serum sample spiked with polydatin and resveratrol (solid line). The analysis were carried out in RP-mode by conditioning a C18 column with a mobile phase consisting of triethanolammonium phosphate (0.1 M, pH= 3.7) and acetonitrile 85:15 (v/v). The column temperature was kept at 40°C. The UV detection wavelength was set at 306nm (for more details see the paragraph 2.3).

Fig. 3 Chromatogram obtained by UHPLC analysis of plasma from volunteer after two hours of treatment with 75 mg polydatin tablet. The analysis was carried out in RP-mode by conditioning a C18 column with a mobile phase consisting of triethanolammonium phosphate (0.1 M, pH= 3.7) and acetonitrile 85:15 (v/v). The column temperature was kept at 40°C. The UV detection wavelength was set at 306nm (for more details see the paragraph 2.3).

Fig. 4 Chromatogram obtained by UHPLC analysis of urine from volunteer after one month of treatment with 75 mg polydatin tablets. The analysis was carried out in RP-mode by conditioning a C18 column with a mobile phase consisting of triethanolammonium phosphate (0.1 M, pH= 3.7) and acetonitrile 85:15 (v/v). The column temperature was kept at 40°C. The UV detection wavelength was set at 306nm (for more details see the paragraph 2.3).

A)



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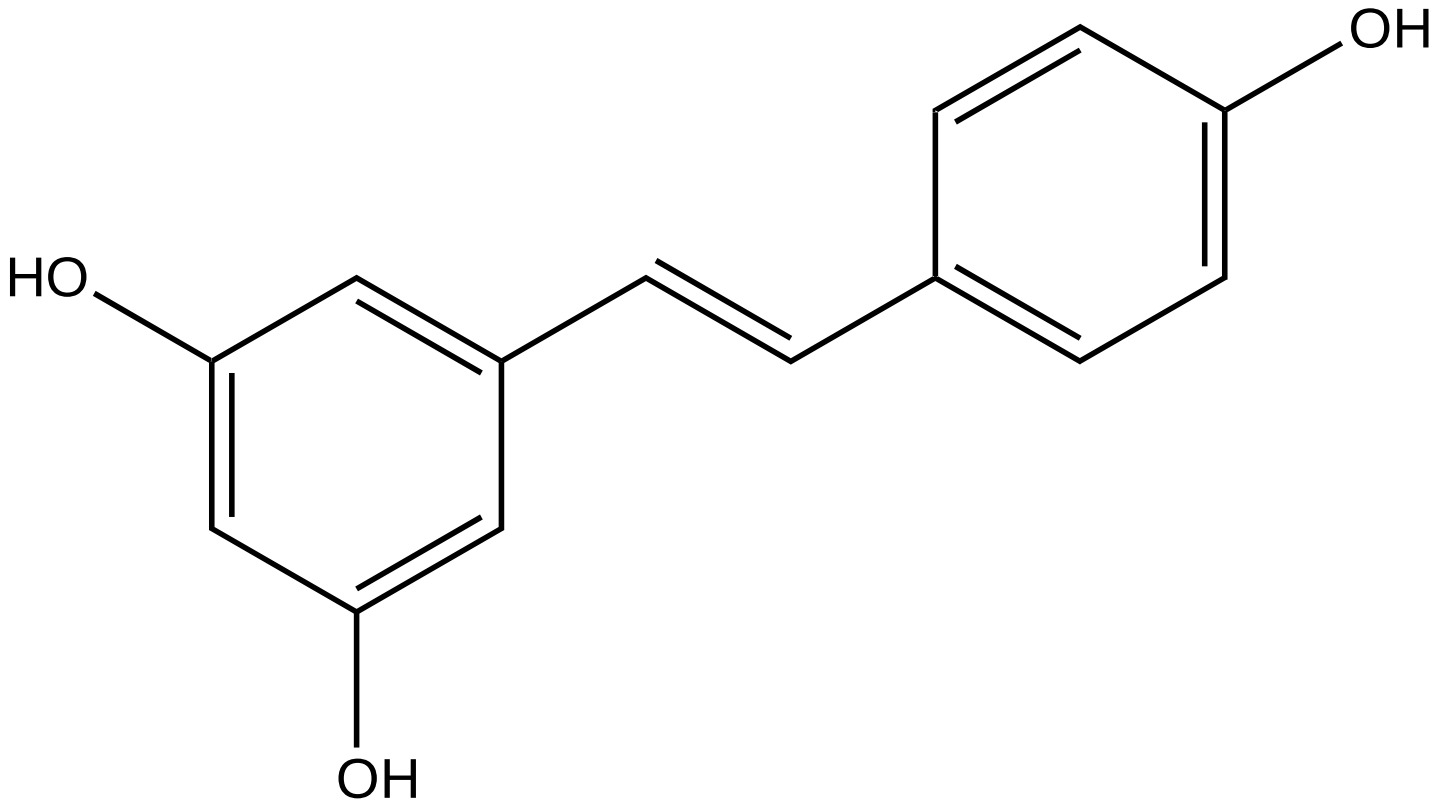

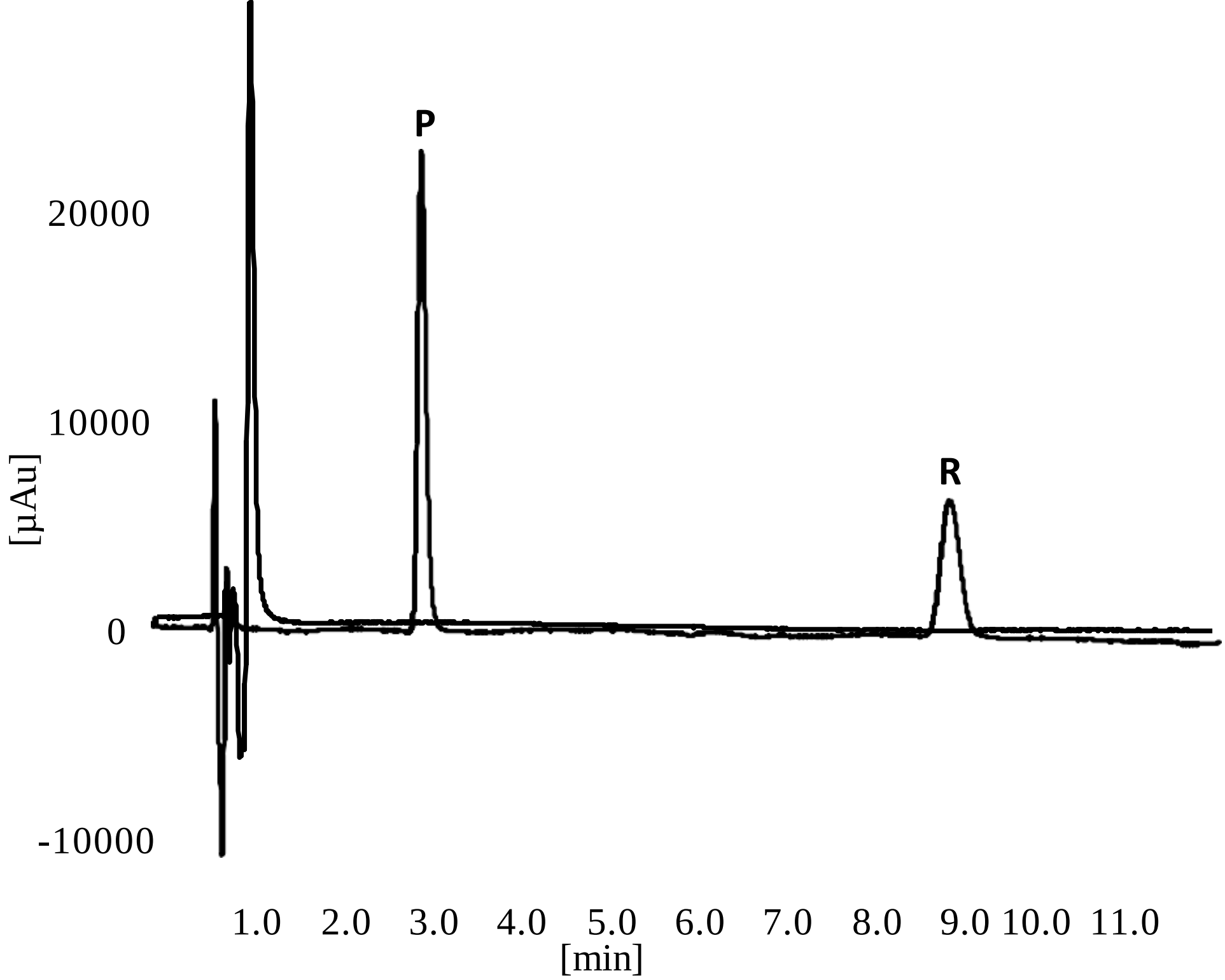
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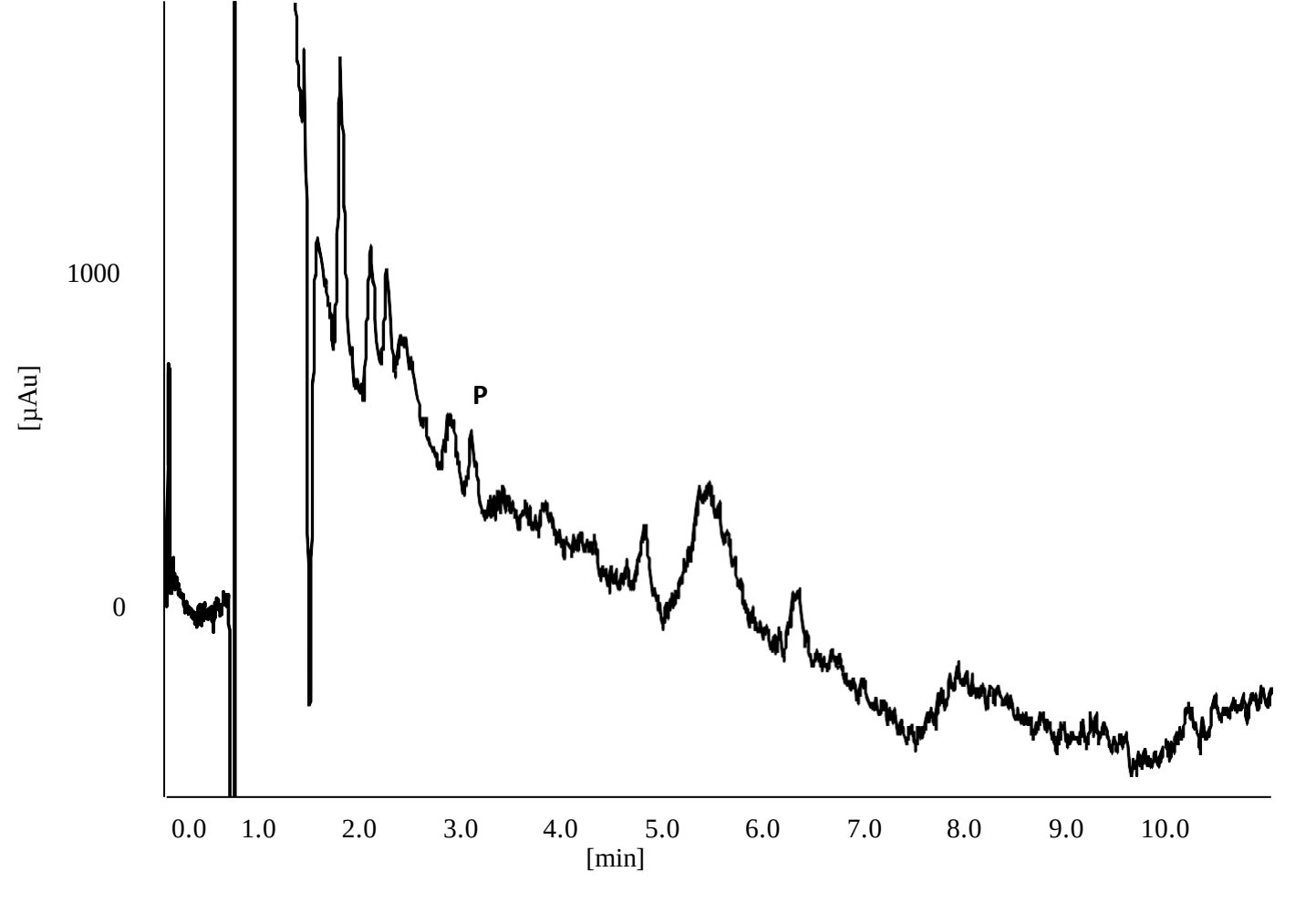
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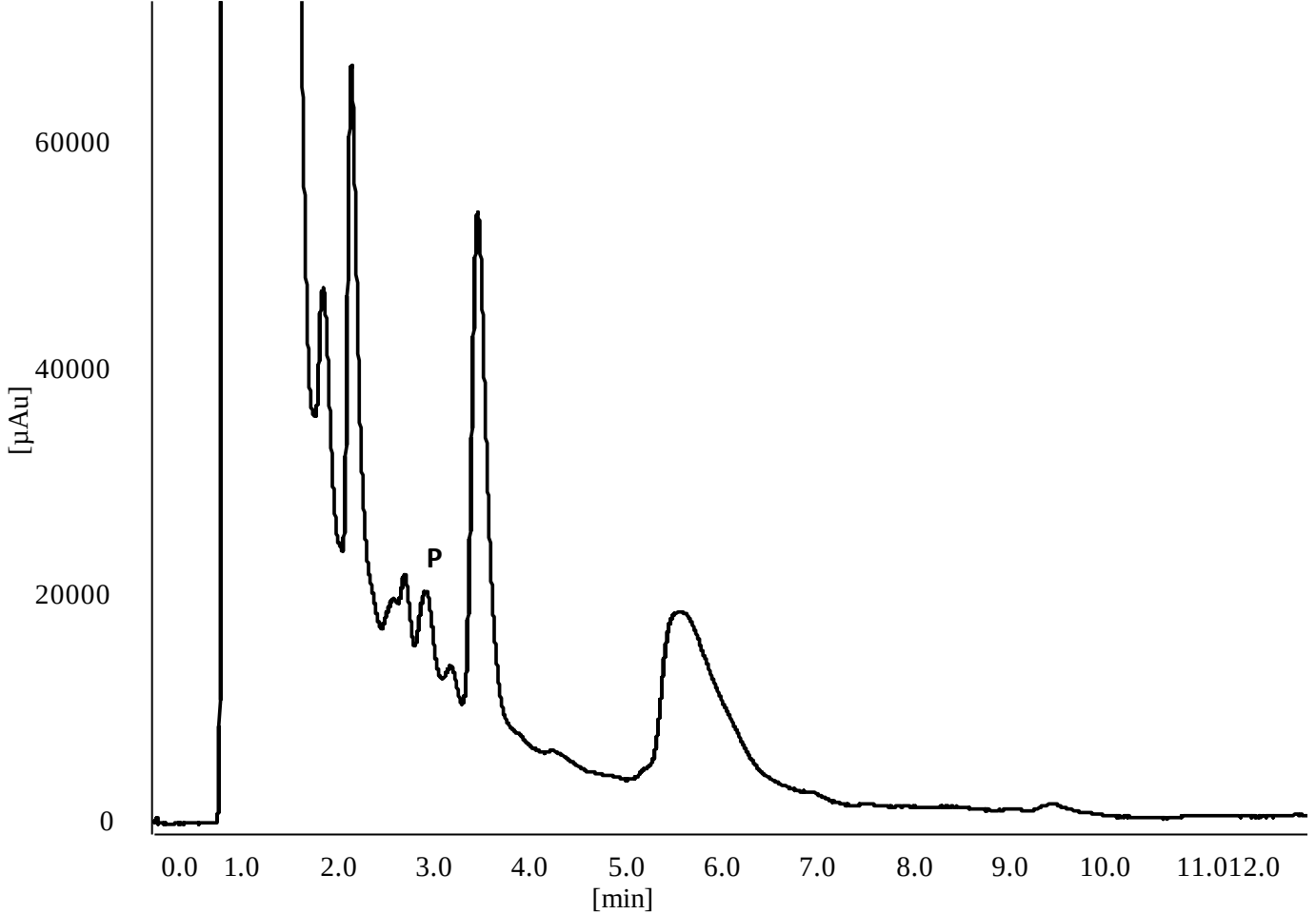
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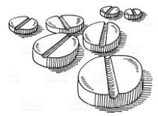
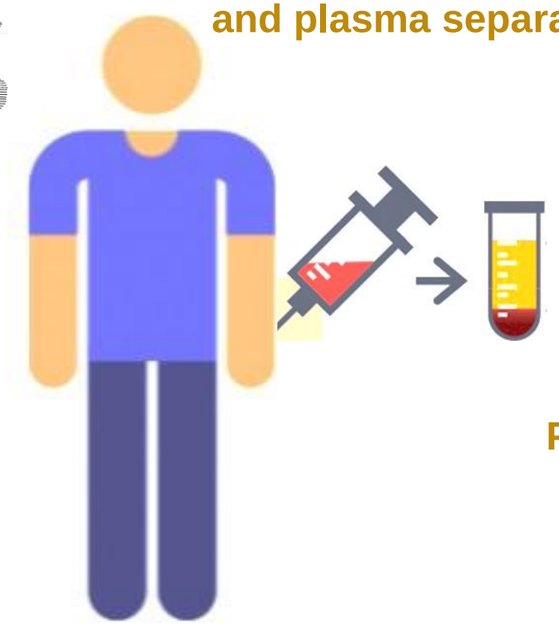
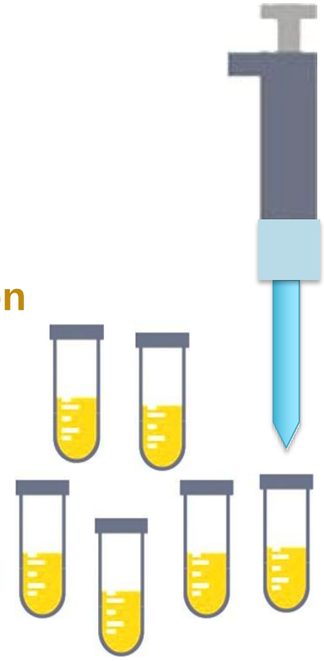
Nutritional supplement uptake



Samples collection and plasma separation

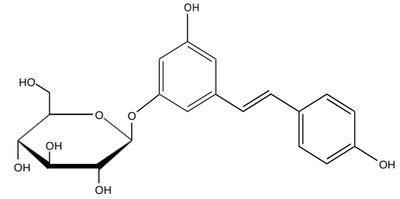




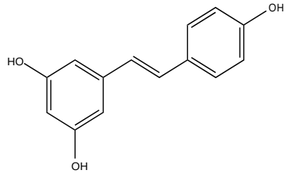
Plasma samples storage and treatment

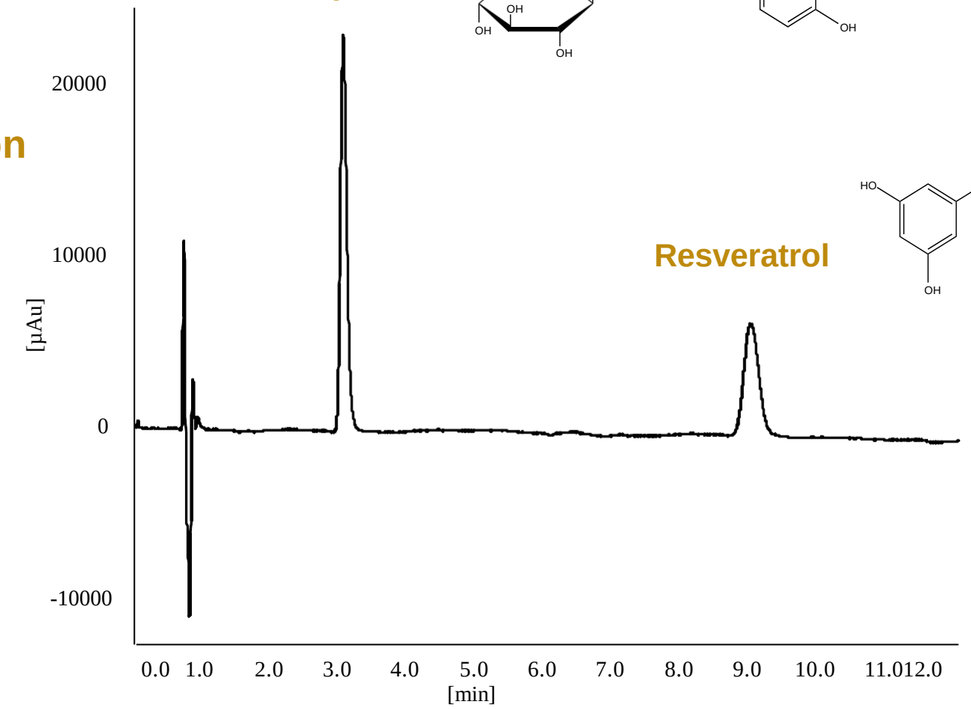
Extraction

Polydatin



Resveratrol





UHPLC analysis

Analyte	Calibration Equation	r^2	Intra-day precision RSD%			Inter-day precision RSD%			LOD (nM in plasma)	LOQ (nM in plasma)	Recovery		
			0.31 µM	1.25 µM	5.00 µM	0.31 µM	1.25 µM	5.00 µM			0.31 µM	1.25 µM	5.00 µM
Polydatin	y=(2355±222,33)x - (184,47±53,48)	0,9967± 0,0016	1,4	0,86	1,93	5,83	0,43	0,19	7,82±0,38	26,06±1,28	94,80± 3,71	93,37± 2,95	97,43± 1,95
Resveratrol	y=(2185±43,13)x + (52,07±27,56)	0,9964± 0,0016	1,03	5,48	1,50	0,93	2,93	2,34	76,28±8,57	254,26±28,57	100,65± 5,06	104,0± 2,96	100,57± 3,48