RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2015, **5**, 17976

Optimization of a liquid chromatography-tandem mass spectrometry method for the quantification of traces of taxanes in a *Corylus avellana* cell suspension medium

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Since the recent discovery of taxol and other taxanes in *Corylus avellana*, this plant species has attracted interest as a potential new source of these compounds. However, its low taxane content in comparison with *Taxus* spp. has restricted research to analytical identification or global quantitation. A feasible and sensitive method based on liquid chromatography-tandem mass spectrometry using a triple quadrupole analyzer was developed for the analysis of taxol and four other taxanes in a *Corylus avellana* cell suspension medium. Taxanes were extracted from the cell culture medium with dichloromethane and analyzed using electrospray ionization and quantified by multiple-reaction monitoring mode. Methanol and matrix-matching calibration curves using docetaxel as the internal standard were analyzed. Linearity was confirmed over the whole calibration range (0.3–2.1 μg mL^{-1}). The inter- and intra-day precision of taxanes ranged from 80% to 120% and the recovery rates were higher than 80%. Limits of detection were between 0.24–38 ng mL^{-1} and the limits of quantification were between 0.8–125 ng mL^{-1}. The low detection and quantitation values obtained allowed us to detect small quantities of the released taxanes (120 ng mL^{-1} of B, 151 ng mL^{-1} of CF and 105 ng mL^{-1} of T), which correspond to about 0.5 ng mL^{-1} of each taxane, in the 20 mL *Corylus avellana* cell suspension culture medium extracted, even at the beginning of the culture. These results were confirmed by high resolution mass spectrometry.

Received 14th January 2015
Accepted 4th February 2015

DOI: 10.1039/c5ra00803d

www.rsc.org/advances

1. Introduction

Taxanes, particularly taxol, are phytochemicals with high added value. Since the discovery of taxol in 1967 in *Taxus brevifolia*, more than 500 natural taxanes have been characterized in different *Taxus* spp., as well as a huge number of synthetic taxol analogues.[1–3]

Taxanes have an important effect against several types of human cancer, including ovarian, breast, head, neck and small cell and non-small cell lung cancers. Their effectiveness in treating AIDS-related Kaposi's sarcoma, lymphoma, and prostate, gastric and bladder cancers has also been recently shown.[4–7] Taxanes are classified as microtubule-interfering agents because they are able to bind with the microtubules and block the cell cycle in the metaphase–anaphase transition, forming highly stable microtubules by suppressing their depolymerization.[8]

Due to the high commercial demand for taxol and its low content in *Taxus* spp., there is a growing interest in exploring new strategies for its production at an industrial level. These include total synthesis, semi-synthetic processes from more abundant taxanes (such as baccatin III, 10-deacetylbaccatin III, 10-deacetyltaxol and cephalomannine), and biotechnological production using cell suspension cultures of *Taxus* spp. or other taxane-producing species such as *Corylus avellana* or fungi.[2] Plant cell cultures constitute an emerging technology for the production of high-value secondary metabolites, which can be scaled up to bioreactor level. They also avoid supply problems associated with the natural sources of these compounds or traditional plant cultivation. An interesting feature of *in vitro* plant cells is their ability to release secondary metabolites into the culture medium, thereby facilitating the *in situ* recovery of the product and promoting a continuous production by the cells. Thus, extracellular availability of the target compounds is preferable to their intracellular extraction from the cells.

To our knowledge, taxol was first found in *C. avellana* trees by Hoffman *et al.* (1998), and since then, the advantages of plant cell cultures have been harnessed to enhance taxol yields in *C. avellana* cell suspensions by different strategies, including elicitation.[9–12]

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Different methodologies to determine taxol and other taxanes are available, most of them using high performance liquid chromatography (HPLC) or Enzyme-Linked ImmunoSorbent Assays (ELISA). HPLC-mass spectrometry (HPLC-MS) has also been used to identify and characterize taxanes in plant samples and cell suspension cultures but not to quantify them, so it was necessary to develop a new reliable methodology to quantify these compounds.[9,13] HPLC-MS/MS is a powerful technique to quantify and determine drugs and metabolites from biological samples, due to its inherent specificity, sensitivity and speed. The HPLC system allows us to separate all the compounds present in the sample extracts, permitting independent MS analysis of the parent ion, which undergoes a selective fragmentation that can be monitored. Moreover, this methodology allows compounds to be detected at trace levels with high reliability.

Although an HPLC-MS/MS method has been described for taxane analysis in *Taxus* spp. plant extracts, no HPLC-MS/MS method has been available to determine the low content of taxol and taxanes found in cell suspension cultures or in *C. avellana* extracts.[14] In addition to the problems arising from matrix complexity, the taxane content in the *C. avellana* tree is ten times lower than in *Taxus* spp.[9] It was therefore necessary to develop a suitable method, including extraction and quantification, able to overcome the matrix issues and detect small quantities of each taxane. The high sensitivity of HPLC-MS/MS methods, especially in multiple-reaction monitoring mode (MRM), indicated it was a suitable technique for taxane analysis in *C. avellana* samples.

In the present work we developed a sensitive HPLC-MS/MS method to quantify five of the most commercially important taxanes: the therapeutic compound taxol, and baccatin III, 10-deacetylbaccatin III, 10-deacetyltaxol and cephalomannine, which can be used for the semi-synthetic production of taxol. Multiple-reaction monitoring mode was used to maximize sensitivity and a positive identification of these compounds by high resolution mass spectrometry (HPLC-ITD-FTMS) was achieved. *C. avellana* cell suspensions were established and the taxanes released into the culture medium were determined and quantified using the reliable analytical method developed. This constitutes the first step in the scale-up process of continuous cultures associated with *in situ* product removal.

2. Experimental

2.1 Chemicals and reagents

All the reagents used were HPLC grade. Methanol, hexane and dichloromethane were purchased from Teknokroma® (Sant Cugat, Barcelona, Spain), acetonitrile LC-MS from Sigma (Madrid, Spain), and MilliQ water was obtained using a Milli-Q system (Millipore). Standards of taxanes, 10-deacetylbaccatin III (DB), baccatin III (B), 10-deacetyltaxol (DT), cephalomannine (CF) and taxol (T), from ChromaDex™ were used to prepare the calibration curves. Docetaxel (DTX), used as an internal standard (IS), was also obtained from ChromaDex™. PVDF syringe filters (13 mm, 0.22 μM pore size) from Teknokroma® were used to filter standard solutions and samples.

2.2 Preparation of standard solutions

Standard stock solutions were prepared in methanol at 50 μg mL^{-1} and kept at −20 °C until use. For the working standard solutions, 10-deacetylbaccatin III, baccatin III, 10-deacetyltaxol, cephalomannine and taxol were dissolved in methanol (MeOH) to obtain seven standard solutions with concentrations in the range of 0.3–2.1 μg mL^{-1}. A stock solution of 50 μg mL^{-1} docetaxel (IS) was prepared in MeOH, and further dilutions were also made in methanol. To carry out the analysis, 5 μL of stock solution was added to samples before the extraction to obtain a final concentration of 1 μg mL^{-1}.

2.3 HPLC-MS/MS conditions

2.3.1 HPLC conditions. The HPLC system consisted of an Agilent 1100 chromatograph fitted with a quaternary pump, a refrigerated autosampler and a UV detector. A Supelcosil™ LC-F 5 μm (25 cm × 4.6 mm) column (Supelco, Bellefonte, USA) was used. The mobile phase consisted of water (A) and acetonitrile (B) with the following gradient (*t* (min), % B): (0, 25), (28, 50), (28.5, 90), (32, 90), (32.5, 25), (40, 25). The flow rate was 1.00 mL min^{-1}, and a 1/3 split was done before the MS. The column was maintained at room temperature and the wavelength was set at 225 nm. Injection volume was 10 μL.

2.3.2 MS/MS conditions. The HPLC system was coupled to a triple quadrupole mass spectrometer API 3000 (AB Sciex, Ontario, CA) fitted with a TurboIon spray source working in positive ion mode and using the following settings: nebulizer gas (N_2) at 9 (arbitrary units), curtain gas (N_2) at 10 (arbitrary units), auxiliary gas (N_2) at 8000 cm^3 min^{-1}, heated at 400 °C, ion spray voltage at +5 kV, CAD gas (N_2) at 5 (arbitrary units), declustering potential DP at +60 V, focusing potential FP at +200 V, entrance potential at +10 V, collision energy at +20 V, and collision cell exit potential CXP at +15 V. MS and MS/MS parameters have been established through infusion experiments using a Harvard syringe pump at 10 μL min^{-1} (individual standard solution at 1 mg L^{-1}) in acetonitrile–water 1 : 1.

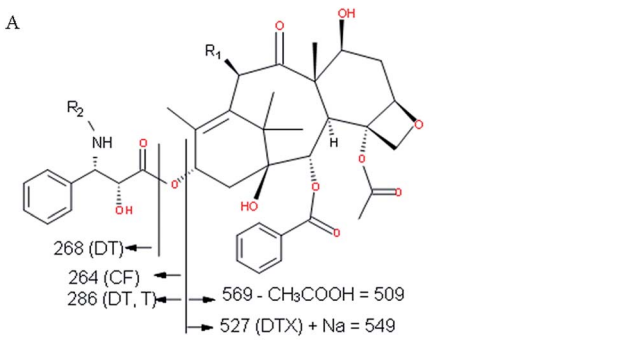
In order to achieve the highest sensitivity, MRM was the acquisition method of choice, all the transitions (Table 1) having a dwell time of 80 ms. Identification and confirmation transitions were determined for each compound. Taxanes were identified by comparing retention times with the standards in MRM trace chromatograms. Data were acquired and analyzed using analyst 1.4.2 software.

2.4 HPLC-ITD-FTMS

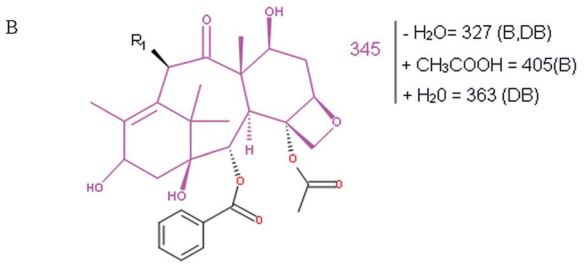
An Accela chromatograph (Thermo Scientific, San Jose; CA,USA) was coupled to an LTQ-Orbitrap Velos instrument (Thermo Scientific, San Jose; CA, USA). A Supelcosil™ LC-F 5 μm (25 cm × 4.6 mm) column (Supelco, Bellefonte, USA) was used and the chromatographic conditions were the same as for the triple quadrupole system. All the analyses were done in positive mode with the following MS conditions: full scan analysis from *m*/*z* 100 to 1100 at 30 000 resolution using the FTMS.

Table 1 Parameters of the HPLC-MS/MS analysis of taxanes: (a) molecular weight (Da), (b) retention time (min); the two MRM transitions for each compound correspond to the identification transition and confirmation transition (*). DP was fixed at 60 V for all compounds

Compound	MW (a)	*m/z*	Molecular ion	CE (V)	MRM	RT (b)
DB	544	545.4	$[M + H]^+$	20	545.4 → 363.1	7.31
			$[M + H]^+$	20	545.4 → 327.4*	
B	586	587.3	$[M + H]^+$	20	587.3 → 405.2	11.88
			$[M + H]^+$	20	587.3 → 327.3*	
DT	811	812.5	$[M + H]^+$	20	812.5 → 286.2	20.50
			$[M + H]^+$	20	812.5 → 268.3*	
DTX	807	808.5	$[M + H]^+$	35	808.5 → 527.0	22.36
		830.5	$[M + Na]^+$	35	830.5 → 549.4*	
CF	831	832.4	$[M + H]^+$	20	832.4 → 509.4	23.02
			$[M + H]^+$	20	832.4 → 264.1*	
T	853	854.5	$[M + H]^+$	20	854.5 → 509.3	25.00
			$[M + H]^+$	20	854.5 → 286.3*	



A) Common structure for taxol, 10-deacetyltaxol, cephalomannine and docetaxel
T: R1 acetoxy group; R2 benzoyl group
DT: R1: hydroxyl group; R2: benzoyl group
CF: R1: acetoxy group; R2: N-Tigloyl group
DTX: R1: hydroxyl group; R2: tert-Butyloxycarbonyl



B) Common structure for baccatin III and 10-deacetylbaccatin III
B: R1: acetoxy group
DB: R1: hydroxyl group

Fig. 1 (a) The common structure for taxol (T), 10-deacetyltaxol (DT), cephalomannine (CF) and docetaxel (DTX). Fragments for each compound are represented. (b) The common structure for baccatin III (B) and 10-deacetylbaccatin III (DB). The fragmentation profile is based on *m/z* 345 (baccatin core) with the described additions and losses.

2.5 Plant material

C. avellana cell suspensions were established from selected white and friable callus induced from hazelnuts. 3 g of calli were inoculated in 30 mL of Murashige and Skoog medium,[15] supplemented with sucrose at 30 g L^{-1}, 2,4-dichlorophenoxyacetic acid 2 mg L^{-1} and kinetin 0.4 mg L^{-1}. The cell cultures were maintained in 200 mL flasks at 25 °C in the dark on an orbital shaker at 110 rpm and were routinely subcultured every 15 days until a fine suspension was obtained, establishing different cell suspension lines. The different cell lines were preliminarily screened for their taxane content. The cultures without taxanes, or with a taxane content below the limit of detection, were used for optimization purposes (calculations of the limit of detection (LOD), limit of quantitation (LOQ), recovery, and matrix effect) and also to obtain the matrix-matching calibration curve, while those containing taxanes were analyzed with the optimized method.

2.6 Sample preparation

Taxanes in the *C. avellana* cell culture medium (20 mL) were extracted using 2 mL of dichloromethane for each 10 mL of medium, vortexed for 2 minutes and sonicated (40 kHz, Branson) for 1 hour. The organic phase was dried with nitrogen gas, dissolved in 250 μL methanol and filtered through a PVDF 0.22 μM filter for subsequent analysis. Samples used for optimization purposes, from cell lines without taxanes, were extracted using the same procedure.

2.7 Quality parameters

2.7.1 Linearity: limits of detection and quantification. Calibration curves were done by plotting the analyte area/IS area *versus* the analyte concentration/IS concentration and weighted using $1/x^2$, obtaining correlation coefficients (r^2) higher than 0.99 and an accuracy in the determination of standard concentration between 80–120%.[16] The standard curve was run on three different days in order to evaluate the stability and linearity of our method. Also, the LOD as the concentration with a signal-to-noise (S/N) of 3 and LOQ as the concentration with a signal to noise (S/N) of 10 were determined.

2.7.2 Precision and accuracy. An intermediate concentration of standard solution (1.2 μg mL^{-1}) was injected eight times to evaluate intra and inter-day accuracy and precision. Quantification was carried out using a calibration curve from the same batch.

2.7.3 Recovery and matrix effect. Recovery and matrix effect were evaluated by the standard addition method, spiking samples pre- and post-extraction, respectively. In the pre-

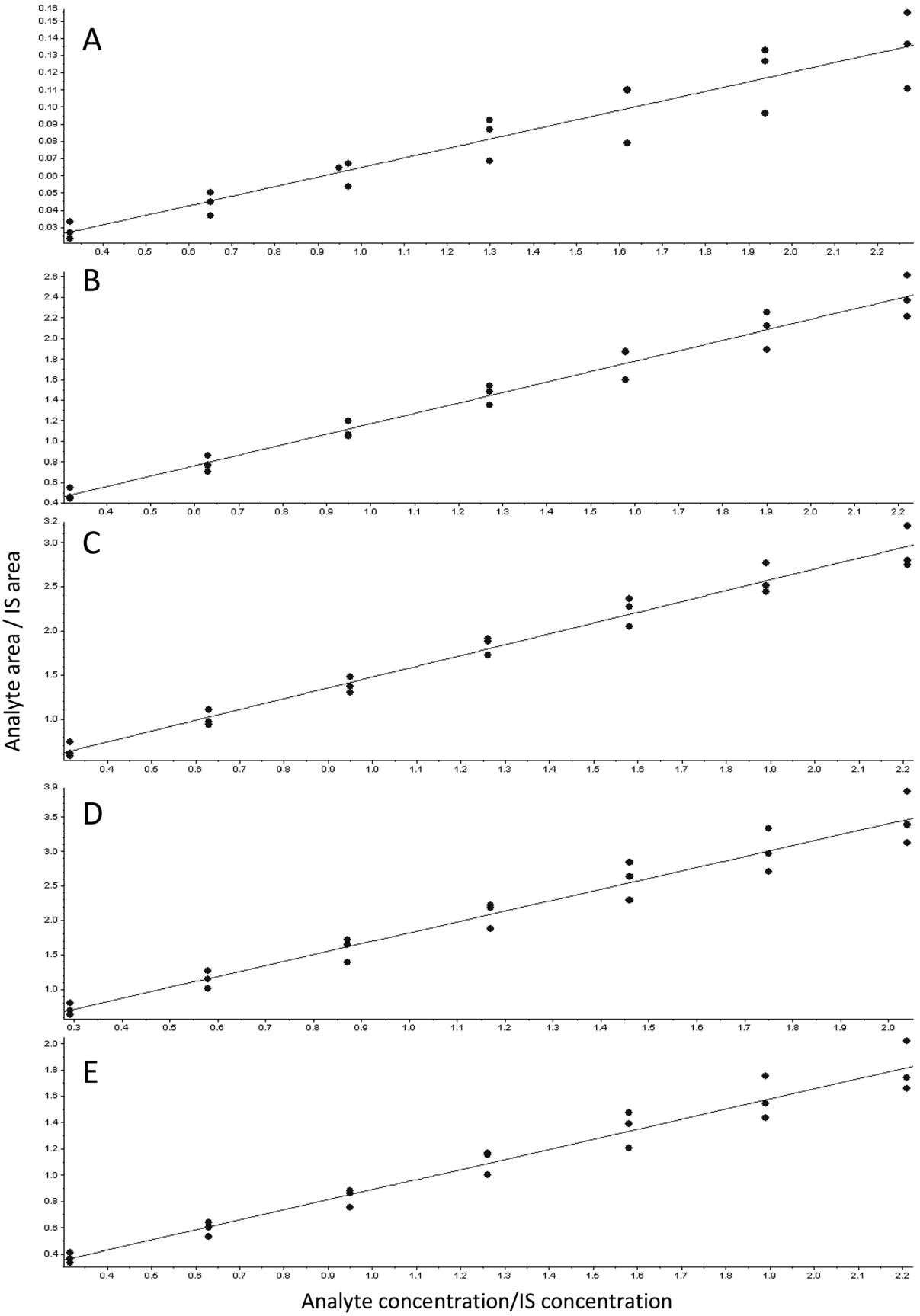


Fig. 2 Standard curves of 10-deacetylbaccatin III (a), baccatin III (b), 10-deacetyltaxol (c), cephalomannine (d) and taxol (e) in the range of 0.3–2.1 μg mL^{-1}. 1 μg mL^{-1} of DTX was added as the internal standard (IS), therefore the analyte area/IS area *vs.* analyte concentration is represented.

Table 2 Mean, relative standard deviation (RSD) and accuracy for each compound regarding the three different standard curves (1, 2, 3). Th.C: theoretical concentration. DB: 10-deacetylbaccatin III. B: baccatin III. DT: 10-deacetyltaxol. CF: cephalomannine. T: taxol

	Intra-day	Th.C. (μg mL^{-1})	Mean (n = 8)	RSD (%)	Accuracy (%)
DB	1	1.30	1.26	1.9	96.9
	2		1.13	4.7	86.6
	3		1.60	1.5	119.0
B	1	1.27	1.29	2.2	101.3
	2		1.12	3.2	88.0
	3		1.40	4.6	110.6
DT	1	1.26	1.28	1.8	101.6
	2		1.10	4.9	87.3
	3		1.38	7.7	109.7
CF	1	1.17	1.20	2.5	102.6
	2		1.03	4.7	88.3
	3		1.42	5.2	119.3
T	1	1.26	1.28	2.9	101.9
	2		1.13	4.1	89.8
	3		1.50	5.1	119.2

Inter-day	Mean (n = 24)	RSD (%)	Accuracy (%)
DB	1.33	15.7	102.2
B	1.27	10.1	99.9
DT	1.25	10.9	98.7
CF	1.22	14.0	104.0
T	1.31	12.7	103.7

extraction, 25 μL of taxanes stock solution in MeOH at 50 μg mL^{-1} was added to 20 mL of the *C. avellana* culture medium to obtain a final concentration of 5 μg mL^{-1}. The taxane extraction was then carried out as described before, and dried down. In the post-extraction addition, the same amount of taxane stock solution in MeOH was added to the dried-down extracts and re-evaporated. 5 μL of the DTX standard solution at 50 μg mL^{-1} was added before the extraction. Samples were resuspended in 250 μL of MeOH.

2.8 Matrix-matching calibration curve

The same range of concentrations as in the standard curve in methanol was added to a non-taxane-containing *C. avellana* cell culture medium extract to obtain the points of the matrix-based calibration curve. The additions were carried out before the extraction. Also, in each case 1 μg mL^{-1} of the DTX as the internal standard was added before the extraction. Taxane extraction was carried out as described before, and the dried-down extracts were resuspended in 250 μL of MeOH. Extractions were carried out in duplicate for each concentration.

3. Results and discussion

Electrospray ionization (ESI) was tested in both positive and negative ion modes, with a higher response found in the former. Therefore, the ESI source in positive ion mode was chosen for taxane detection. The mass spectra were recorded in the range of m/z 100–1000 amu. The main ions obtained from the standards were as follows: DB: m/z 545.4 $[M + H]^+$, B: m/z 587.3 $[M + H]^+$, DT: m/z 812.5 $[M + H]^+$, CF: m/z 832.4 $[M + H]^+$, T: m/z 854.5 $[M + H]^+$, and DTX: m/z 808.5 $[M + H]^+$. Also, m/z 830.5 $[M + Na]^+$ was observed for DTX. Since declustering potential (DP) and collision energy (CE) played a significant role in generating the final MRM mode, both parameters were optimized in order to have the maximum signal for each analyte in infusion experiments (DP 60 V and CE 25–30 V). Identification transitions, which were defined as those with highest intensity, and the confirmation transitions obtained are shown in Table 1.

McClure *et al.* (1992) and Kerns *et al.* (1994) reported the fragmentation pattern of taxol and 19 natural taxanes, describing the fragment ion characteristic of the paclitaxel core as m/z 509. The target compounds showed lateral chains with different m/z fragments: m/z 286 in the case of T, m/z 286 and m/z 268 for DT, and m/z 264 for CF. Also, m/z 327 was thought to correspond to the baccatin III core after water loss.[13,17] Similarly, we hypothesize that m/z 405 corresponds to the baccatin III core with a carboxylic acid substitution, and m/z 363 to the baccatin III core with water incorporation. The Multiple Reaction Monitoring (MRM) transitions for DTX are m/z 808.5 → 527.0, described by Corona *et al.* (2011), and m/z 830.5 → 549.4 corresponding to the Na^+ adduct.[18] Fig. 1 depicts the common structure for each compound, and the different fragments used for their identification. Although some m/z fragments were already described, only a descriptive fragmentation was usually reported, and there are no studies available combining these data with the evaluation of the quality parameters and with the quantification using a matrix-matching calibration curve.

3.1 Linearity

The standard curve analysis before the use of the IS showed an increasing angle of slope during the three days (data not shown). To solve this problem, we searched for a suitable IS. Taking into account that an IS should be a compound of the same chemical class as the analytes but with different molecular weight and different MRM transitions, we chose DTX as an appropriate IS to determine the taxanes in our samples, since it fulfilled the aforementioned requirements. Moreover, as a synthetic analogue, it was not present in our samples.

The linearity of the chromatographic method was determined using a mixed standard solution of DB, B, DT, CF and T at seven concentrations in the range of 0.3–2.1 μg mL^{-1} supplemented with 1 μg mL^{-1} of the IS DTX. The graph therefore represents the analyte area/IS analyte area *vs.* analyte concentration/IS concentration (Fig. 2). The regression equation and the correlation coefficients of each compound in each standard curve were determined. R^2 was higher than 0.997 for all the compounds in the three different standard curves, showing a good linearity over the measured range and allowing a good extrapolation of our data. Weighted least squares linear regression by a factor of $1/x^2$ resulted in an accuracy range of 80–120% in terms of RSD.[16]

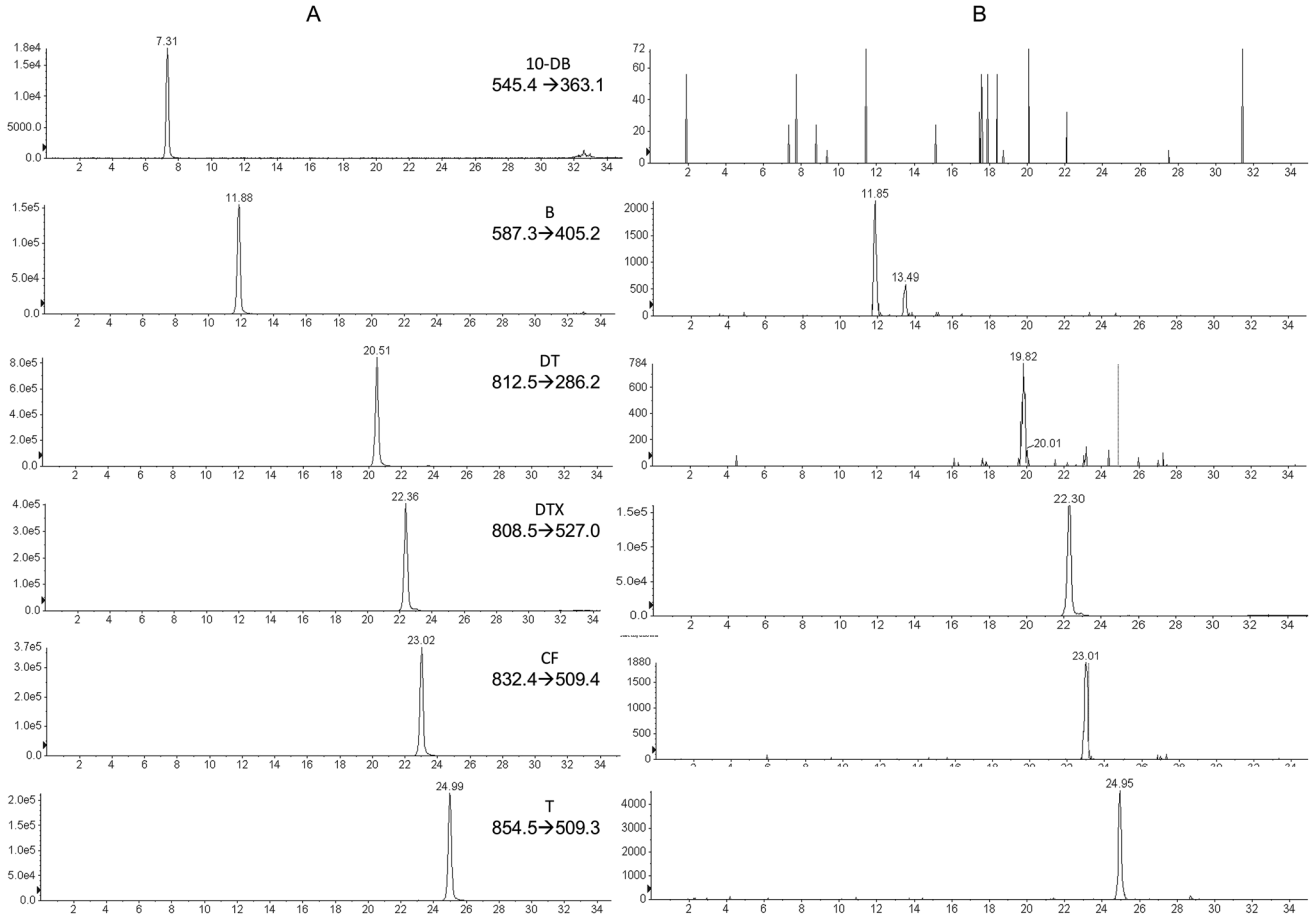


Fig. 3 Identification chromatograms for taxane traces, (a) standard solution at 1.2 μg mL^{-1} with 1 μg mL^{-1} DTX as IS. (b) *C. avellana* sample with 1 μg mL^{-1} DTX.

3.2 Limits of detection and quantification

Limits of detection and quantification were determined taking into account the matrix effect. With this aim, *C. avellana* samples from non-taxane-containing cell lines were considered as blank samples and therefore spiked to obtain a concentration of 0.3 μg mL^{-1}. $S/N = 3$ and $S/N = 10$ were used to determine LOD and LOQ, respectively. The LOD was 37.6 ng mL^{-1} for DB, 2.42 ng mL^{-1} for B, 0.24 ng mL^{-1} for DT, 0.53 ng mL^{-1} for CF and 0.79 ng mL^{-1} for T. The LOQ was also determined for all the compounds, being 125.5 ng mL^{-1} for DB, 8.0 ng mL^{-1} for B, 0.80 ng mL^{-1} for DT, 1.8 ng mL^{-1} for CF and 2.7 ng mL^{-1} for T. No taxanes were found in the blank sample, although they may have been present below the detection limit.

3.3 Precision and accuracy

Intra- and inter-assay precision and accuracy were determined by assaying eight replicates of an intermediate concentration (1.2 μg mL^{-1}). The calculated concentrations from the appropriate standard curve in each analytical run were used to obtain the relative standard deviation (RSD). Accuracy was assessed as the percentage of RSD from the theoretical concentration. These parameters are described in Table 2. Intra-day repeatability reached a maximum RSD of 7% and accuracy ranged between 82–119% RSD. Inter-day repeatability RSD was always lower than 15% and accuracy was between 98–104%. Taking into account all these parameters, we developed a reliable and reproducible method, with a repeatability not exceeding 15% RSD and an accuracy always within the range of 80–120%. Additionally, the RSD of the retention times was calculated for all the runs and was always lower than 1% for all the compounds, again illustrating the chromatographic robustness of the method.

3.4 Recovery accuracy and matrix effect

Many parameters can influence sample analysis, two of the most important being recovery and the matrix effect. Recovery is based on the amount of product collected after the extraction method. In complex matrices, such as plant samples, an extraction method providing pure compounds is extremely difficult to develop, so recoveries are usually low. On the other hand, the matrix effect, ion suppression or ion enhancement are due to other compounds in the sample interfering with the detection of the target compounds. The taxane standard solution was added to 5 μg mL^{-1} *C. avellana* culture medium before the extraction to analyze recovery,

and then after the extraction to analyze the matrix effect. Calculated concentrations were compared with the theoretical concentration added. Recovery by this methodology was 100% for DB, 86.2% for B, 84.6% for DT, 82.9% for CF and 87.5% for T. These percentages were obtained taking into account the calculated suppression for each compound. The results showed a matrix effect of −44.4% for DB, 49.9% for B, 42.6% for DT, 43.4% for CF and 42.9% for T in comparison with the responses achieved when injecting the methanolic solution. The high efficiency of the extraction method and the sensitivity of the system allowed us to detect our compounds despite a certain degree of suppression in our analytical process.

3.5 Matrix-matching calibration curve

The same range of taxane concentrations as in the standard curve were added to the *C. avellana* cell culture medium extracts from non-taxane-containing cell lines to obtain a calibration curve. Linearity of the calibration curve was analyzed for all the compounds, showing an r^2 of 0.987 for DB, 0.993 for B, 0.992 for DT, 0.986 for CF and 0.984 for T. Weighting of $1/x^2$ was applied to obtain an accuracy of 80–120%, while real accuracies were 92.5–114%. Fig. 3 shows the MRM transitions (identification) for standard compounds (A) and for a sample of *C. avellana* cell culture medium from a producer cell line (B), detecting and quantifying B, CF and T. The ion ratios between the confirmation and identification transition were calculated and compared with those obtained in the standard, allowing us to confirm the presence of the compound when the difference was less than 20%. The *C. avellana* medium extract was quantified using the matrix-matching calibration curve, obtaining a concentration of 120 ng mL^{-1} for B, 151 ng mL^{-1} for CF and 105 ng mL^{-1} for T. Taking into account the original volume of medium extracted (20 mL), concentrations of 0.5 ng mL^{-1} for B, 0.63 ng mL^{-1} for CF and 0.44 ng mL^{-1} for T were produced by *C. avellana* cells and released into the medium. These results had been confirmed in the extract using HPLC-ITD-FTMS by comparing their retention times with those of standards previously injected in the same conditions and by the exact mass $[M + H]^+$ of the ions present in the FTMS spectra with an accuracy of ±2 mDa.

It is therefore possible to affirm that *C. avellana* is a taxane-producing plant, although the concentrations obtained were very low in comparison with those obtained in *Taxus* spp.[19] The analysis was carried out at day 3 of the cell suspension culture because of the low taxane concentration obtained at that point. The developed method is therefore sufficiently sensitive to detect taxane production from the beginning of the culture, when the production is usually extremely low. Razaei *et al.* (2011) reported a taxol content of 13 μg L^{-1} in the medium of a *C. avellana* cell suspension culture growing in control conditions at day 12 by HPLC-UV analysis.[12] Bemani *et al.* (2013) reported 16 μg L^{-1} of extracellular taxol at day 14, quantified by HPLC-UV and confirmed by HPLC-MS, but no information about taxol production in the early stages of culture and quantitation by HPLC-MS/MS has been previously reported.[20]

4. Conclusions

We have developed and optimized a reliable and sensitive HPLC-MS/MS method to determine five of the most commercially important taxanes (taxol, baccatin III, 10-deacetylbaccatin III, 10-deacetyltaxol and cephalomannine) present at a nanogram level in a *Corylus avellana* cell suspension culture medium. The culture media of *C. avellana*, a potential new source of taxanes, is of particular interest, as it facilitates the *in situ* recovery of the product and promotes a continuous production by the cells. In addition, extracellular availability of the target compounds is crucial for scaling up the process.

This new analytical procedure is able to detect secreted taxanes even at the beginning of the culture, when the production is usually extremely low, and allows the production profile to be followed throughout the assay. To the best of our knowledge, this is the first reported application of a liquid chromatography-tandem mass spectrometry method to quantify individual taxanes in *Corylus avellana*, and to evaluate linearity, precision, accuracy, recovery, and the matrix effect, as well as to develop a matrix-matching calibration curve to quantify more precisely the lower concentration of these compounds.

Acknowledgements

We thank the Centres Científics i Tecnològics of the Universitat de Barcelona (CCiTUB) for their help. This research has been supported by a grant from the Spanish MEC (BIO2011-29856-CO2-01) and a grant from the Catalan Government (2014SGR215). A. Gallego was supported by a fellowship from the University Pompeu Fabra.

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