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Short communication

Enhanced method performance due to a shorter chromatographic run-time in a liquid chromatography–tandem mass spectrometry assay for paclitaxel

Kjell A. Mortier [a], Alain G. Verstraete [b], Guo-Fang Zhang [a], Willy E. Lambert [a,*]

[a] *Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium*
[b] *Department of Clinical Biology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium*

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Abstract

Liquid chromatography–tandem mass spectrometry (LC–MS–MS) is often performed in a high-throughput environment. Unfortunately, with atmospheric pressure ionization (API) techniques, shorter run-times or reduced sample clean-up often result in ion or matrix suppression, which can lead to erroneous results. The present work on the analysis of paclitaxel compares ion suppression, sensitivity and linearity of a high-throughput LC–MS–MS method (0.8 min run-time, method B) to a method with increased separation (2.0 min run-time, method A). An atmospheric pressure chemical ionization (APCI) interface was used for both methods. The high-sample-throughput method uses an increased amount of organic solvent in the mobile phase (isocratic, 85% versus 70% of methanol) and a higher flow-rate (600 μl/min versus 400 μl/min). As a result, internal standard (docetaxel) and target analyte (paclitaxel) co-elute, close to, although separated from the solvent front. Ion suppression of both methods was evaluated by comparing pure standard to plasma and plasma containing a vehicle. Sensitivity and linearity were compared by injecting matrix matched calibration samples with both methods. Ion suppression by the vehicle Cremophor EL led to poor data quality for the standard method (A), while for the short method (B), ion suppression was compensated for by the co-eluting internal standard. The short method showed similar linearity but increased sensitivity by at least a factor five. This work provides a strategy to compensate for ion suppression without the use of labeled internal standards. In addition, a better sensitivity and a shorter run-time are noted.

Keywords: Sensitivity; Paclitaxel; Docetaxel

1. Introduction

Ion suppression in atmospheric pressure ionization (API) for liquid chromatography coupled to mass spectrometry (LC–MS) is now well documented throughout the literature [1–3]. It is caused by the presence of material other than the target compound during the ionization process and may result in altered ionization efficiency of that compound. It has been reported that electrospray is more susceptible to ion suppression than atmospheric pressure chemical ionization (APCI) [4], although this assumption cannot be generalized completely [5]. General strategies to tackle ion suppression involve an improved LC separation efficiency and/or a more selective sample clean-up [6–8]. These strategies clearly lead to a lower sample throughput and a higher workload. Ideally, isotopically labeled internal standards can be used to compensate for suppression (or enhancement) effects. These compounds co-elute (i.e. enter the ion source) with the analyte of interest and experience similar ionization conditions [2,8]. However, these compounds are often not available or cost-prohibitive.

This work describes how ion suppression was compensated for by decreasing the run-time from 2.0 to 0.8 min. The mobile phase of the shortened, isocratic LC method contained more organic solvent and could be delivered at a higher flow-rate, resulting in nearly complete co-elution of the analyte, paclitaxel (PAC), and the internal standard (I.S.), docetaxel. The performance of both methods is compared

* Corresponding author. Tel.: +32-9-264-81-35; fax: +32-9-264-81-83.
E-mail address: willy.lambert@ugent.be (W.E. Lambert).

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with a focus on sensitivity, linearity and ion suppression. Especially the influence of the hydrophobic Cremophor EL on the paclitaxel ionization is considered. Cremophor EL is the vehicle used for the delivery of Taxol, the commercial formulation of paclitaxel. After administration, it remains present in plasma at high concentrations for a significant period [9]. Recently, ion suppression by a number of polyethylene glycol (PEG) based vehicles was reported [10]. Therefore, we considered it important to include an evaluation of ion suppression of paclitaxel by Cremophor EL, in view of future pharmacokinetic studies with Taxol.

2. Experimental

2.1. Standards and reagents

Stock solutions of paclitaxel and docetaxel (Sigma–Aldrich, Bornem, Belgium) were prepared by accurately weighing 10 mg of powder and dissolving this in 10 ml of methanol. Stock solutions were stored at −20 °C. From the stock solution, working standards were prepared by dilution with methanol to the final concentration of 0.01, 0.025, 0.1, 0.25, 1, 2.5, and 10 μg/ml, respectively. Cremophor EL (Sigma–Aldrich) stock solution was prepared by dissolving 3.2 g in 100 ml of water. Dilutions were made in water to 0.5, 1, 2, 4, and 8 mg/ml. These solutions are called the Cremophor EL working standards.

2.2. Sample preparation

Sample preparation consisted of a liquid–liquid extraction (LLE) procedure with methyl *tert*-butyl ether (MTBE). [11] To 500 μl of Na_2EDTA plasma, 100 μl of the internal standard solution was added. Calibrators were prepared by adding 100 μl of paclitaxel working standards to the plasma. A 3.0 ml aliquot of MTBE was added and after 10 min on a rotor device and centrifugation (5 min, 1600 × *g*), the upper organic layer was transferred evaporated. Samples were reconstituted with 200 μl of the mobile phase of which 10 μl was injected. For samples spiked after sample preparation, 100 μl of the working standard and 100 μl of a 0.5 mM aqueous acetic acid solution were used for reconstitution.

2.3. Liquid chromatography

The HPLC system consisted of a fully equipped Agilent 1100 configuration (Agilent Technologies, Palo Alto, CA, USA). Isocratic elution at ambient temperature was conducted on a Phenomenex Synergi Max RP (2.00 mm × 75 mm, 4 μm particles) with guard column. For method A (standard LC method) the mobile phase, methanol–water (70:30, v/v) with 0.5 mM acetic acid, was delivered at a flow rate of 400 μl/min. Method B (fast LC method) used methanol–water (85:15, v/v) with 0.5 mM acetic acid pumped at 600 μl/min.

2.4. Mass spectrometry

An API 2000 triple quadrupole instrument from Applied Biosystems (Foster City, CA, USA) equipped with a heated nebulizer interface (APCI) was operated in the positive mode. Multiple reaction monitoring was performed with 200 ms dwell times. The transitions for paclitaxel were *m/z* 854 > 105 and for docetaxel *m/z* 808 > 226 at unit resolution for both the first and the third quadrupole.

2.5. Method comparison: sensitivity, linearity and robustness to ion suppression

Method sensitivity was compared by analyzing blank Na_2EDTA plasma spiked with 100 μl of the working solutions and subjected to the LLE procedure. Signal-to-noise ratios (S/N) of the plasma extracts are compared for the evaluation of sensitivity. Linearity of both methods is also compared by analyzing these samples and plotting peak area against concentration.

For the evaluation of robustness regarding ion suppression, three sets each of five samples were prepared at the 2.5 ng on-column (oc) level (Table 1). A first set of samples (SET 1, standard) was used to monitor the response and variation of the ratio PAC/I.S. in the absence of matrix or vehicle material. A second set (SET 2, plasma) showed the effect of residual matrix material on the ionization. A third set of samples (SET 3, plasma + 0.5 ml Cremophor EL working standards) was used to verify the effect of the plasma extract plus vehicle material (clinically relevant and

Table 1
Sample preparation for evaluation of robustness to ion suppression

SET 1 ($n = 5$)	SET 2 ($n = 5$)	SET 3 ($n = 5$)
Standard	LLE plasma	LLE plasma + Cremophor EL working standards (5 ≠ increasing concentration)
	500 μl Na_2EDTA plasma LLE with 3 ml M*t*BE Evaporate M*t*BE	500 μl Na_2EDTA plasma + 500 μl Cremophor EL working standards LLE with 3 ml M*t*BE Evaporate M*t*BE
	Redissolve residue with:	Redissolve residue with:
100 μl PAC–I.S. 500 ng/ml 100 μl 0.5 mM acetic acid 10 μl injected	100 μl PAC – I.S. 500 ng/ml 100 μl 0.5 mM acetic acid 10 μl injected	100 μl PAC–I.S. 500 ng/ml 100 μl 0.5 mM acetic acid 10 μl injected

concentrations [12]) on the ionization of paclitaxel. Any discrepancy in signal stability is believed to result from ion suppression by matrix or vehicle material.

3. Results and discussion

Chromatograms obtained from the analysis of extracted plasma samples by both methods are displayed in Fig. 1. The peak height of paclitaxel is increased with method B. This as a result of a higher percentage of organic phase (a decreased retention on the LC-column and higher ionization efficiency) and a higher flow-rate. Table 2 depicts the S/N values for the lower points of the calibration curves. At least a five fold increase in sensitivity is noted for method B at the lower concentrations.

For the evaluation of linearity, matrix matched calibration curves (1.25–125 ng oc for method A; 0.125–12.5 ng oc for method B) were injected with both methods (Table 2). The correlation coefficient (r) is similar for both methods, indicating that linearity is comparable.

Robustness to ion suppression was evaluated by injecting the three sets of samples (standard, plasma extract, and plasma + Cremophor EL extract), each set consisting of five samples. The samples from SET 1, consisting of standard solutions, were re-injected after SET 2 and 3 to monitor a possible cross-over effect from previous runs. These sets are called SET 1 bis and SET 1 tris, respectively. All samples were evaluated with method A and B. Results (area and ratio of PAC) for SET 3 and SET 1 tris with method A are low compared to previously analyzed sets (Table 2). We believe this is the result of ion suppression by the hydrophobic vehicle Cremophor EL (added in SET 3) of which the effect even persists in the subsequent set (SET 1 tris) through delayed elution from the column. The baseline separation of PAC and internal standard in method A also increased the variation of the PAC/I.S. ratio, as ionization conditions can be different for both compounds, with PAC being more suppressed than the I.S. as a result of an earlier elution. Due to co-elution of PAC and I.S. in method B, ionization suppression (or enhancement) of both compounds is more similar, which counteracts the variation to a certain extent (area ratio of different sets is more similar). In addition, with a higher amount of organic solvent in the mobile phase it is likely that hydrophobic interferences like Cremophor EL are eluted faster.

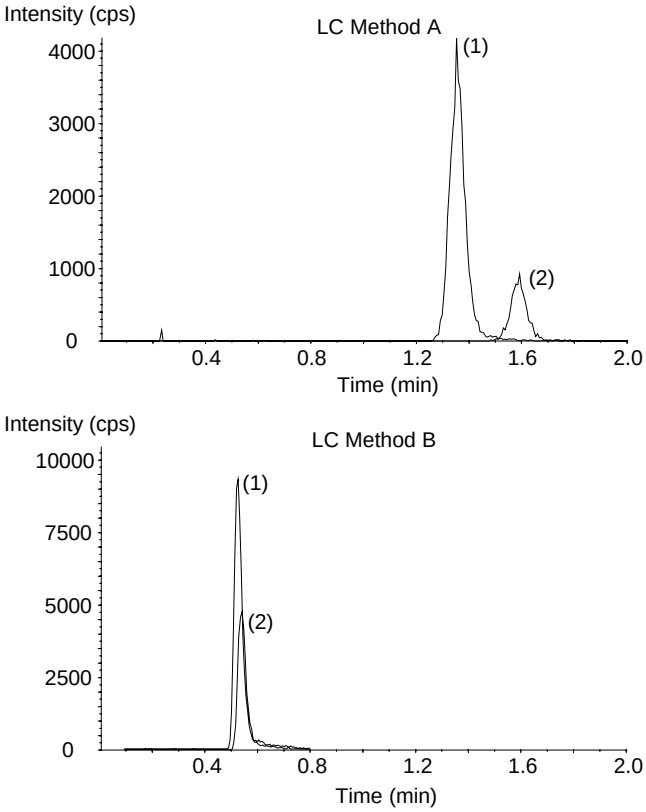

Fig. 1. Comparison of chromatograms obtained by method A and B for blank plasma spiked to 10 ng paclitaxel/ml. (1) Paclitaxel and (2) internal standard: docetaxel.

With these experiments, we have demonstrated that short LC methods do not necessarily compromise the data quality due to e.g. ion suppression. Some limitations are related to this approach. First of all, analyte and internal standard

Table 2
Signal-to-noise ratio's of the lower points of the calibration curve, calibration curve equation (range: 1.25–125 ng oc for method A; 0.125–12.5 ng on-column for method B) with correlation coefficient (r) for plasma extracts with both methods and area ratio's (with R.S.D. values) for the different sets of the ion suppression experiment

	Matrix matched calibrators				
	S/N values				Calibration curve
Amount injected (ng oc)	0.05	0.125	0.5	Equation	r
Method A	2	7	17	$y = 2.88x - 6900$	0.9992
Method B	14	45	130	$y = 2.55x + 325$	0.9994
	Area ratio (R.S.D., %) of the sets				
Ion suppression	SET 1	SET 2	SET 1 bis	SET 3	SET 1 tris
Method A	0.98 (3.4)	1.01 (1.2)	1.04 (2.7)	0.91(5.5)	0.90 (3.5)
Method B	0.83 (4.7)	0.85 (5.1)	0.91 (4.4)	0.86 (6.0)	0.86 (2.2)

should have very similar chemical and physical properties, especially regarding polarity. This is a prerequisite to obtain co-elution and similar behavior during the ionization process. A close structural analogue is therefore strongly recommended. Another limitation involves the simultaneous analysis of multiple compounds. Metabolites or degradation products would be very difficult to monitor by the same method, due to differences in polarity. Therefore, successful application of this approach is restricted to target analysis of one compound co-eluting with its internal standard. Bearing in mind these limitations, our work provides a strategy to maintain data quality in a high throughput environment, without the use of a labeled internal standard.

4. Conclusion

During method development, the run-time of a method was significantly reduced from 2 to 0.8 min, by increasing the flow-rate and amount of organic solvent in the isocratic LC method. As a consequence, co-elution of analyte and internal standard was obtained. This loss in chromatographic resolution did not negatively affect the method performance. On the contrary, a higher sensitivity was observed and ion suppression was more efficiently compensated for by the co-eluting internal standard. This work provides a way of achieving a sensitive and reproducible high throughput analysis for one target compound without a labeled internal standard being available.

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