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Matrix effect in LC-ESI-MS and LC-APCI-MS with off-line and on-line extraction procedures

Sandrine Souverain, Serge Rudaz, Jean-Luc Veuthey*

Laboratory of Pharmaceutical and Analytical Chemistry, School of Pharmacy, University of Geneva, 20 Bd d'Yvoy, 1211 Geneva 4, Switzerland

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Abstract

Matrix effect on mass spectrometry response was investigated with commercially available electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) sources coupled with a single quadruple mass spectrometer. A post-column infusion system was used to observe the MS signal alterations of methadone, selected as the model compound, in plasma. For this purpose, two sample preparation procedures were tested: (1) conventional off-line sample preparations with liquid–liquid extraction (LLE), solid-phase extraction (SPE) and protein precipitation (PP) with perchloric acid (PA) and acetonitrile (ACN) and (2) on-line SPE with two different extraction columns packed with a large particle support (LPS) and with restricted access material (RAM), respectively. Whatever the sample preparation procedures, APCI source appeared to be less liable to matrix effect than ESI source. Among the different off-line sample preparations, LLE was the most efficient extraction procedure. With other techniques, MS signal was affected not only by endogenous material but also by procedure introduced compounds. Moreover, on-line SPE-LC-MS configuration exhibited matrix effects, which depend on the API source and the extraction support.

Keywords: Matrix effects; Methadone; Off-line sample preparation; On-line solid-phase extraction

1. Introduction

In the last 10 years, atmospheric pressure ionisation (API) sources have widely contributed to the success of liquid chromatography coupled to mass spectrometry (LC-MS) for the fast analysis of pharmaceutical compounds in biological matrices [1–6]. In order to achieve high throughput analysis, automated, simplified and rapid sample preparation procedures, such as protein precipitation (PP) and solid-phase extraction (SPE) performed on 96-well plates, as well as on-line extraction techniques coupled to fast liquid chromatography using short analytical columns and fast gradients, recently appeared [7,8].

Electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) are the most currently used API sources. In spite of the fact that they are based on different ion formation mechanisms [9–15], both are considered as "soft ionisation" sources inducing preferentially the formation of the de-protonated or protonated molecule without fragmentation. MS signal suppression or enhancement effects have been widely reported in the literature when complex matrices are analysed [16–32]. This undesirable phenomenon, termed matrix effect, is generally not reproducible or repeatable between various sample batches or even samples and, thus, compromises a quantitative analysis. Different models have been proposed to explain the mechanism of matrix effect in ESI [22,30,33] but it is usually accepted that it results from the ionisation competition between the different species eluted from the column. Thus, matrix effects have to be investigated during the early development of a LC-ESI-MS. For this purpose, several approaches were described, such as the use of spiked biofluids after extraction [34] and the post-column infusion systems [21,24,27–29,35–37]. To overcome matrix effects, different strategies can be performed, such as a selective sample preparation [17,18,23,27,35] or/and an efficient chromatographic separation [16,18,20,25–27,31].

APCI is clearly less investigated than ESI source but it is generally reported that the former is less susceptible to matrix effect because ionisation takes place in the gas phase

* Corresponding author. Tel.: +41 22 379 63 36; fax: +41 22 379 68 08.
E-mail address: jean-luc.veuthey@pharm.unige.ch (J.-L. Veuthey).

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[16,17,19,29,32,38,39]. Moreover, it was demonstrated that source design could have a relevant effect on signal response [39].

In this paper, the matrix effect was evaluated with commercially available ESI and APCI sources with a single quadrupole mass spectrometer. Four conventional off-line extraction procedures and three SPE coupled on-line to LC-MS were evaluated for the extraction of human plasma. Off-line liquid–liquid extraction (LLE) and SPE procedures, already optimised for the LC-UV analysis of methadone enantiomers [40] and two protein precipitations were performed.

The three extraction supports selected to assess the matrix effect in the column-switching configuration were a restricted access media (RAM) and two large particle supports (LPS) already used for the analysis of pharmaceutical compounds in biological fluids [41]. All extraction procedures were investigated on the methadone (MTD) MS signal, using a post-column infusion system for both API sources.

2. Experimentals

2.1. Chemicals

Methadone hydrochloride (MTD) was obtained from Hanseler AG (Herisau, Switzerland). Perchloric acid (PA), hydrochloric acid, acetic acid, potassium dihydrogen phosphate, potassium hydroxide, isoamyl alcohol, sodium carbonate, isopropanol and ammonium formate were purchased from Sigma (Buchs, Switzerland). Ammonia was obtained from Reactolab S.A. (Servio, Switzerland). Acetonitrile (ACN), hexane, dichloromethane and formic acid were obtained from SDS (Peypin, France). Water was provided by a Milli-Q Gradient A10 water purifier system from Millipore (Bedford, MA, USA). Human blank plasma were obtained from the Centre de Transfusion of Geneva Hospital (Geneva, Switzerland).

2.2. Off-line sample preparations

Four sample preparation procedures were used: SPE on a mixed mode Bond Elut Certify sorbent (130 mg sorbent mass, 3 mL column volume) from Varian (Harbor City, USA), LLE with a mixture of hexane: isoamyl alcohol and protein precipitation (PP) with PA and ACN. All procedures were applied to six human plasma from different origins and to purified water. For all off-line preparations, 10 μL of extracted plasma was injected in the LC-MS post-column infusion system (see Section 2.4.1).

2.2.1. Solid-phase extraction and liquid–liquid extraction

One millilitre of blank plasma was extracted by SPE and LLE with the procedures already described elsewhere [40].

2.2.2. Protein precipitation

2.2.2.1. Perchloric acid precipitation. Five hundred microlitres of an aqueous perchloric acid solution at 6% (v/v) was added to 500 μL of blank plasma. Samples were vortex mixed and centrifuged for 5 min at 6000 × *g*. Five hundred microlitres of the supernatant was diluted with 250 μL of ammonium formate 1 M, the pH of the sample was adjusted to approximately 3.6.

2.2.2.2. Acetonitrile precipitation. One thousand microlitres of ACN was added to 500 μL of blank plasma. After vortex mixing, the sample was centrifuged for 5 min at 6000 × *g*. Nine hundred microlitres of supernatant was evaporated to dryness under a gentle stream of nitrogen at 37 °C. The residue was reconstituted with 300 μL of mobile phase constituted of a mixture ACN:water (35:65, v/v) at 0.1% formic acid.

2.3. On-line sample preparations

For all on-line preparations, 50 μL of supernatant was injected in the column-switching system (see Section 2.4.2).

2.3.1. Direct injection

Blank plasma was diluted 1:1 with water. After vortex mixing, samples were centrifuged for 5 min at 6000 × *g*.

2.3.2. After protein precipitation

One thousand microlitres of ACN was added to 500 μL of blank plasma. After vortex mixing, the sample was centrifuged for 5 min at 6000 × *g*.

2.4. Liquid chromatography-mass spectrometry

All experiments were performed on an Agilent Series 1100 LC system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, a binary pump and a six-port switching valve. An additional Agilent Series 1100 LC isocratic pump was included in the system for the column-switching configuration. The LC system was coupled to an Agilent Series 1100 MSD single quadrupole (Agilent Technologies) equipped with orthogonal ESI or orthogonal APCI sources. MS parameters were optimised for each ionisation source and are reported in Table 1. Nitrogen was used both as nebulising and drying gas. For both sources, MS detection was conducted in the single ion monitoring mode (SIM), the

Table 1
MS parameters optimised for ESI and APCI sources

	ESI parameters	APCI parameters
Nebulisation pressure (psi)	25	10
Flow rate of drying gas (L min^{-1})	11	5
Temperature of drying gas (°C)	300	350
Capillary voltage (V)	3000	3500
Corona discharge (μA)	–	3
Temperature of vaporizer (°C)	–	350

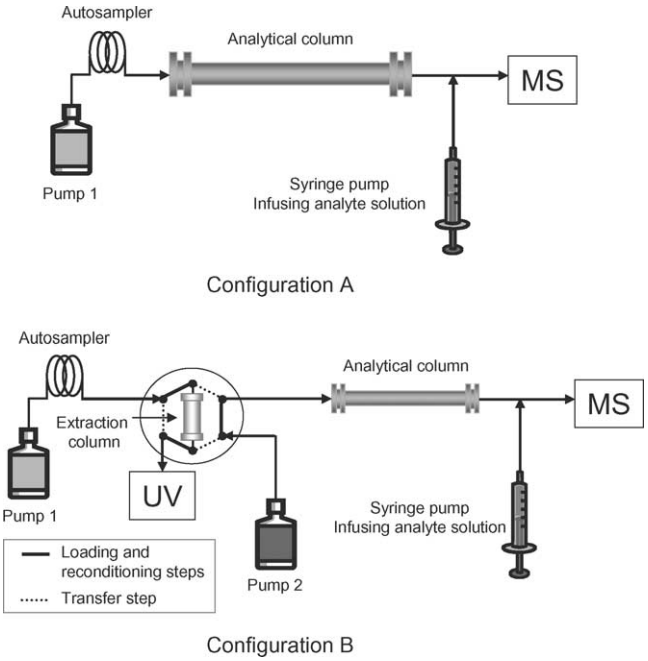

Fig. 1. Post-column infusion systems: (A) off-line sample preparation configuration and (B) SPE coupled on-line to LC-MS configuration.

protonated molecule of MTD at 310 u was recorded with a skimmer voltage optimised at 65 V.

The Chemstation software suite A.09.03 (Agilent Technologies) was used for instrument control, data acquisition and data handling. To achieve analyte post-column infusion, a Harvard Model 22 syringe pump (South Natick, MA, USA) was used.

2.4.1. Off-line post-column infusion configuration

For the off-line configuration, a conventional LC set-up with a post-column infusion system was used, as shown in Fig. 1A. Ten microlitres of mobile phase, water and blank plasma extracted by off-line sample preparations was injected in a chromatographic Purospher STAR RP-18e column (55 mm × 2 mm i.d., d_p 3 μm) from Merck (Darmstadt, Germany). The mobile phase was constituted of 0.1% (v/v) formic acid in water:ACN (65:35) (v/v) delivered at a flow rate of 300 μL min^{-1}. A MTD solution in the mobile phase at 2 μg mL^{-1} was infused post-column with the syringe pump at a flow rate of 2 μL min^{-1}.

2.4.2. On-line post-column infusion configuration (column-switching)

Fig. 1B represents the column-switching post-column infusion system with a Purospher STAR RP-18e column (55 mm × 22 mm i.d., d_p 3 μm) from Merck as analytical column. Selected extraction supports were (1) Oasis HLB column (50 mm × 1 mm i.d., d_p 30 μm) from Waters Corp. (Milford, MA, USA), (2) Cyclone column (50 mm × 1 mm i.d., d_p 50 μm) from Cohesive Technologies Inc. (Franklin, MA, USA) and (3) LiChrospher RP-4 ADS (25 mm × 2 mm i.d., d_p 25 μm) from Merck. The syringe pump infused the MTD solution at 2 μg mL^{-1} at a flow rate of 2 μL min^{-1}.

Table 2
Switching time in column-switching configuration according to the extraction supports used

Extraction supports	Oasis HLB	Cyclone	LiChrospher RP-4 ADS
Loading step (min)	0–1	0–1	0–4
Transfer step (min)	1–3	1–3	4–8
Reconditioning steps (min)	3–10	3–10	8–10

Fifty microlitres of mobile phase, blank plasma diluted 1:1 with water or precipitated was injected on the extraction supports with a mobile phase constituted of 0.1% (v/v) formic acid in water:ACN mixture (95:5) (v/v) (Fig. 1B). The loading flow rates were 4 mL min^{-1} with both LPS supports and 0.8 mL min^{-1} with RAM. After the extraction step, the switching valve was switched. The analyte was transferred from the extraction support to the chromatographic column with a mobile phase constituted of 0.1% (v/v) formic acid in water:ACN mixture (65:35) (v/v) at a flow rate of 300 μL min^{-1}. After the transfer step, the valve was switched to its original position for pre-column reconditioning. The switching time for each extraction support is summarized in Table 2.

3. Results and discussion

3.1. Off-line sample preparation

According to Bonfiglio et al. [35], a post-column infusion system was used with the continuous infusion of analyte solution between the analytical column and the MS source. In order to ensure that matrix effect was really due to endogenous components and not to the procedure, water samples treated by the different extraction procedures were also injected in the LC-MS post-column infusion system with ESI and APCI sources (Fig. 1A).

Initial experiments demonstrated that plasma samples from different origins exhibited homogenous MS responses whatever the sample preparation procedure and the source (data not shown).

Fig. 2 represents post-column infusion chromatograms obtained by LC-ESI-MS for all the off-line extraction procedures. The identical and stable profiles of mobile phase, water and plasma extracted by LLE indicate that there is no matrix effect due to endogenous or extraneous components elution (Fig. 2A). With the SPE procedure, the signal was suppressed for the first minute for the extracted plasma and water samples (Fig. 2B). Therefore, the signal suppression was not correlated to matrix components but to the extraction procedure itself. According to the chromatographic system and to their low retention, these interferences seemed polar. A similar phenomenon was observed for protein precipitation procedures with PA (Fig. 2D). This could be explained

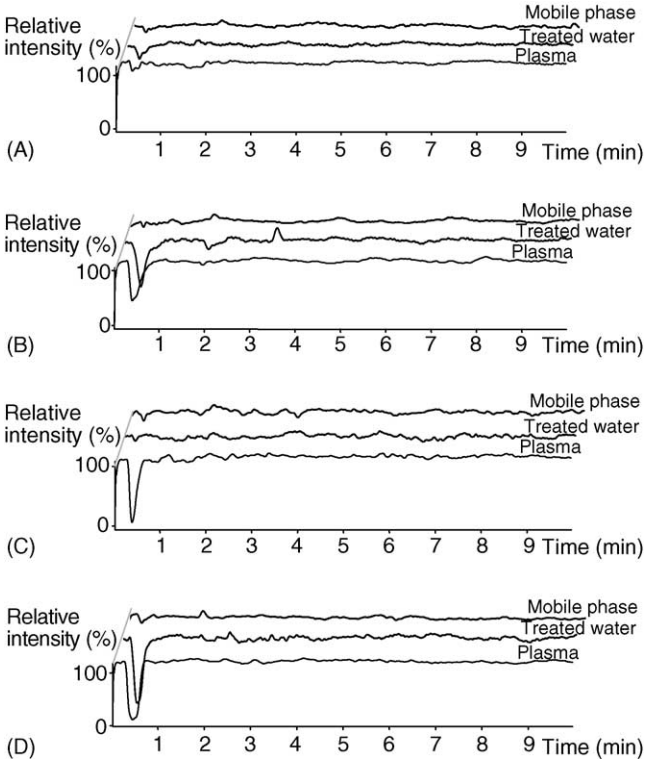

Fig. 2. LC-ESI-MS post-column infusion chromatograms for the four off-line extraction procedures: (A) LLE; (B) SPE; (C) PP with ACN; and (D) PP with PA. Analytical conditions as described in Section 2.

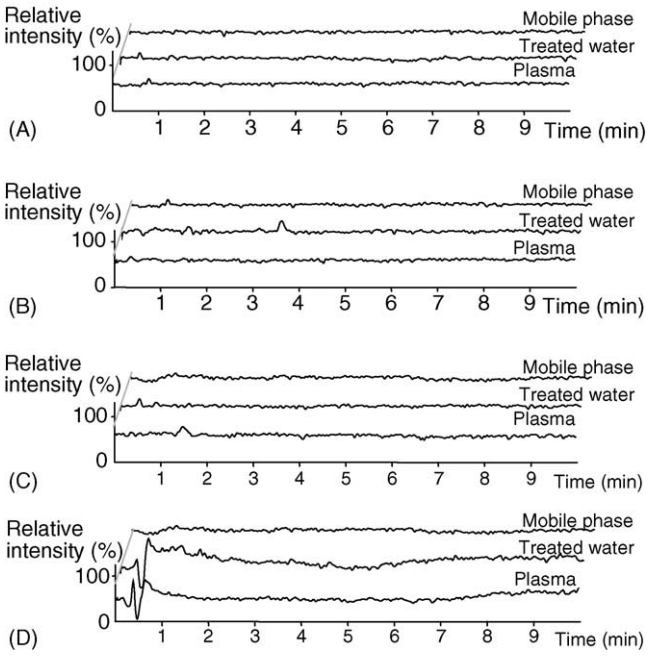

Fig. 3. LC-APCI-MS post-column infusion chromatograms for the four off-line extraction procedures: (A) LLE; (B) SPE; (C) PP with ACN; and (D) PP with PA. Analytical conditions as described in Section 2.

by large amount of salts in the samples, such as perchlorate or formate salts which can induce MTD signal suppression. However, some other polar compounds could also induced matrix effect, which could be masked by the high amount of salts introduced with the sample preparation procedure. With ACN precipitation, the MS signal suppression was only observed for plasma samples, while a stable MS response was obtained for mobile phase and treated water (Fig. 2C). Therefore, the signal suppression was certainly, in this case, induced by substances in the matrix.

With the APCI source, no MS signal suppression occurred for all off-line extraction procedures except protein precipitation with PA (Fig. 3). However, this phenomenon was less marked with APCI than with ESI source.

Whatever the off-line sample preparations, the MS signal suppression was recorded during the first minute of chromatograms. However, given MTD eluted outside the matrix effect window (e.g. with retention time higher than 1 min) under developed chromatographic conditions, the performances of the methods were not compromised.

3.2. On-line sample preparation

As shown in Fig. 1B, a column-switching configuration including a post-column infusion set-up was also used. Three samples were injected in the column-switching configuration: loading mobile phase, blank plasma diluted 1:1 with water (direct injection) and blank plasma precipitated with ACN. By comparing the MS chromatograms observed for the different samples, it is possible to attribute a MS signal alteration to protein material. However, the presence of acetonitrile in the solvent sample could be responsible of removing some interferents during the loading step even if a dilution of the sample was probably obtained under high flow rate conditions. Six plasma samples coming from different origins gave the same MS profile (data not shown). Fig. 4 presents the post-column infusion ESI-MS chromatograms for each sample injected on the three extraction supports (Oasis HLB, Cyclone and LiChrospher RP-4 ADS). With Oasis HLB support, a stable MS signal was observed (Fig. 4A). In all cases, the signal suppression at 1.5 min was due to the column-switching configuration and was stabilized after 30 s (t = 2 min).

The injection of a blank plasma diluted 1:1 with water and loaded onto a Cyclone support led to a significant signal suppression during the transfer step (Fig. 4B). The MS signal returned to its initial level after 3 min. This suppression was not observed with the injection of precipitated plasma and loading mobile phase. Therefore, it was attributed to the elution of endogenous material (such as proteins) previously retained by the extraction support during the loading step. This hypothesis was confirmed by the column pressure increase between injections (about 2 bar per injection).

With the restricted access material (RAM), similar ESI-MS chromatograms were recorded for the injection of loading mobile phase and precipitated plasma. Given the low flow rate

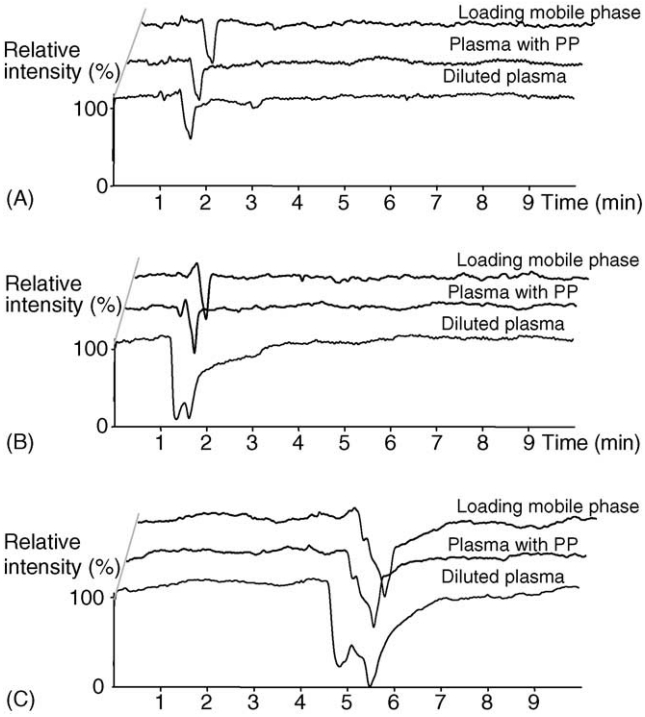

Fig. 4. SPE-LC-ESI-MS post-column infusion chromatograms for the three extraction supports: (A) Oasis HLB (50 mm × 1 mm i.d., d_p 30 μm); (B) Cyclone (50 mm × 1 mm i.d., d_p 50 μm); and (C) LiChrospher RP-4 ADS (25 mm × 2 mm i.d., d_p 25 μm). Analytical conditions as described in Section 2.

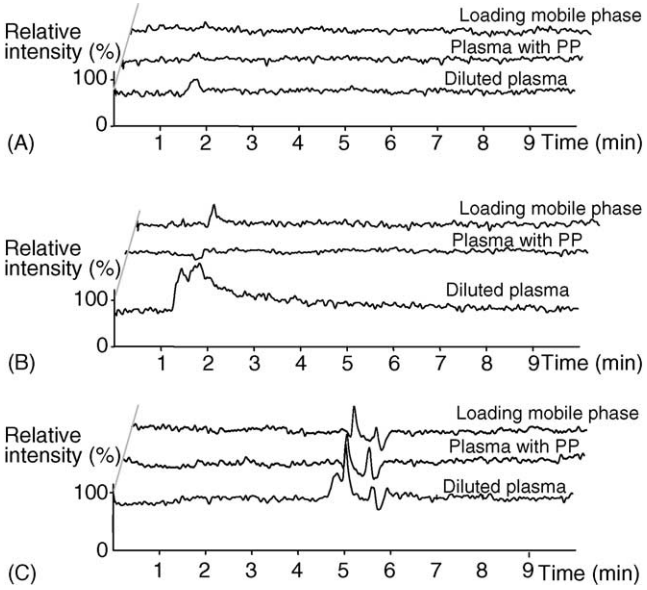

Fig. 5. SPE-LC-APCI-MS post-column infusion chromatograms for the three extraction supports: (A) Oasis HLB (50 mm × 1 mm i.d., d_p 30 μm); (B) Cyclone (50 mm × 1 mm i.d., d_p 50 μm); and (C) LiChrospher RP-4 ADS (25 mm × 2 mm i.d., d_p 25 μm). Analytical conditions as described in Section 2.

used to perform the extraction step, the valve was switched after 4 min. After 2 min, the ESI-MS signal intensity reached its initial level. With the injection of the diluted plasma, important signal suppression occurred for 3 min. Although a matrix effect was observed with the LiChrospher RP-4 ADS pre-column, no pressure increase was recorded in the analytical column.

The APCI-MS chromatograms for the three extraction supports and for each injected sample are reported in Fig. 5. As previously described, a stable signal was recorded with the Oasis HLB support (Fig. 5A). The signal suppression previously observed with ESI source was negligible in APCI-MS. A significant APCI-MS signal enhancement occurred with the direct injection of plasma on a Cyclone pre-column (Fig. 5B). The stable APCI-MS chromatograms obtained with the loading mobile phase and the precipitated plasma demonstrated that endogenous materials were partially retained on this support and induced a matrix effect. An increase of pressure was also recorded in the analytical column after direct injection of plasma. A negligible alteration of APCI-MS signal was recorded for each sample injected in RAM support at the beginning of the transfer step (Fig. 5C). However, the APCI-MS signal alteration was slightly more important for the direct injection of diluted plasma.

For all on-line SPE procedures, the MS signal suppression or enhancement was observed during the transfer step. However, since MTD eluted after the transfer step, no matrix effect was observed for all developed methods.

4. Conclusion

Matrix effects were compared with ESI and APCI sources for the analysis of a model drug (i.e. methadone) in human plasma.

APCI was less susceptible to matrix effect than ESI source. Among the off-line sample preparations, LLE was found to be the most efficient extraction procedure with both API sources. SPE and PP with PA led to plasma samples containing polar extraneous interferences susceptible to induce signal suppression with ESI source. A similar ESI-MS signal suppression at the beginning of the chromatogram was observed for PP with ACN and was mainly due to the elution of endogenous compounds. For on-line SPE-LC procedures, the two LPS demonstrated different extraction behaviours: while no matrix effect was observed for Oasis HLB, Cyclone exhibited a relevant alteration of MS signal. A less significant matrix effect was also observed for RAM.

Because MTD eluted after the matrix effect window, which arisen at the beginning of chromatograms, the performances of the developed methods were not compromised.

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